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MERKEL CELL CARCINOMA

EPIDEMIOLOGICAL STUDY WITH SPECIAL REFERENCE TO POLYOMAVIRUS AND VASCULAR FACTORS IN PATHOGENESIS

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ACADEMIC DISSERTATION

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To my family

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LIST OF ORIGINAL ARTICLES

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I Kukko H, Böhling T, Koljonen V, Tukiainen E, Haglund C, Pokhrel A, Sankila R, Pukkala E. Merkel cell carcinoma – nationwide population-based epidemiologic study with clinical series of 181 cases. Submitted.

II Kukko HM, Koljonen VSK, Tukiainen EJ, Haglund CH, Böhling TO. Vascular invasion is an early event in pathogenesis of Merkel cell carcinoma. Mod Pathol 2010;23:1151-6.

III Sihto H*, Kukko H*, Koljonen V, Sankila R, Böhling T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst 2009;101:938-45.
*equal contribution

IV Kukko H, Koljonen V, Lassus P, Tukiainen E, Haglund C, Böhling T. Expression of vascular endothelial growth factor receptor-2 in Merkel cell carcinoma. Anticancer Res 2007;27(4C):2587-9.

V Kukko H, Koljonen V, Lassus P, Tukiainen E, Haglund C, Böhling T. Expression of endostatin in Merkel cell carcinoma. Anticancer Res 2007;27(4C):2583-6.

LIST OF ABBREVIATIONS

AEOIU	Asymptomatic, Expanding rapidly, Immune suppression, Older than 50 years, Ultraviolet-exposed site
AIDS	acquired immune deficiency syndrome
AJCC	American Joint Committee on Cancer
APUD	amine precursor uptake and decarboxylation
BCC	basal cell carcinoma
BKV	BK virus (first discovered in a patient whose initials were B.K.)
BVI	blood vascular invasion
CD-31	a cell adhesion molecule named CD-31 (also known as PECAM-1)
ChrA	chromogranin A
CK-20	cytokeratin 20
CLL	chronic lymphatic leukemia
CLND	completion lymph node dissection
Flk-1	fetal liver kinase 1
Flt-1	fms-like tyrosine kinase
HIV	human immunodeficiency virus
IHC	immunohistochemistry
JCV	JC virus (first discovered in a patient whose initials were J.C.)
KDR	kinase domain region
LCA	leukocyte common antigen
LVI	lymphovascular invasion
LYVE	lymphatic vessel endothelial hyaluronic acid receptor
MC	Merkel cell
MCC	Merkel cell carcinoma
MCPyV	Merkel cell polyoma virus
MSKCC	Memorial Sloan-Kettering Cancer Center
NCAM	neural cell adhesion molecule
NFP	neurofilament
NSE	neuron specific enolase
PCR	polymerase chain reaction
PECAM	platelet endothelial cell adhesion molecule
Prox-1	prospero homeobox 1
PUVA	psoralen + ultraviolet A
RSR	relative survival ratio
RT	radiotherapy
SCC	squamous cell carcinoma
SCLC	small cell lung carcinoma

SCM	small cell melanoma
SEER	Surveillance, Epidemiology, and End Results Program
SNB	sentinel node biopsy
SYP	synaptophysin
TTF-1	thyroid transcriptase factor 1
UV	ultraviolet
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

ABSTRACT

Background and purpose: Merkel cell carcinoma (MCC) is a rare malignancy of the skin, and its incidence is reported to be rising. MCC usually displays rapid growth with frequent lymph node and systemic metastases. Integration of Merkel cell carcinoma polyomavirus (MCPyV) genome to the tumor genome was recently found to be common in these cancers. The purpose of this study was to review all MCC cases in Finland based on the Finnish Cancer Registry and to calculate the incidence of Merkel cell carcinoma in Finland, and further, to present a clinical series of patients whose tumor samples were available for re-evaluation. The aim was also to investigate the clinical consequences of MCPyV genomic integration and to examine vascular invasion and the expression of vascular endothelial growth factor-2 (VEGFR-2) and endostatin in primary MCC, and their prognostic value.

Methods: All cases with a notation of Merkel cell carcinoma or small cell carcinoma of skin during 1979–2008 (n=295) were obtained from the Finnish Cancer Registry and reviewed. The incidence was calculated from this data. If available, the formalin-fixed paraffin-embedded tissue samples of patients diagnosed with MCC during 1979–2004 (n=207) were tracked and collected to create a clinical series. The tissue samples were available from 193 patients, and after re-confirmation of the diagnosis, a total of 181 cases were reviewed in more detail for clinical course, treatment and survival. Some of these tissue samples were further analyzed for vascular invasion (n=126) by immunohistochemistry using vascular endothelial markers CD-31 and D2-40, and for MCPyV (n=114) by using a polymerase chain reaction (PCR) and quantitative PCR. Immunohistochemical analyses were performed for the expression of VEGFR-2 (n=21) and endostatin (n=19). These findings were compared to the clinical data and disease outcome.

Results: The age-adjusted incidence of MCC (/100,000) in Finland is 0.11 for men and 0.12 for women when adjusted to the world standard population. The mean age at diagnosis was 75.9 years, and the majority of the patients were women. The most common site of the primary tumor was the head and neck. No additional benefit was found from a wide margin (≥ 2cm) compared to margin of 0.1–0.19 cm, but intralesional excision was more often associated with local recurrence. None of the patients with Stage I-II who had received postoperative radiotherapy to tumor bed had a local recurrence. The 5-year relative survival among men was 36% (95% CI 20-54%), and among women 69% (CI 56-82%).

Intravascular tumor cells were observed in 93% of the 126 samples studied. The majority had only lymphovascular invasion. The tumors lacking invasion were

significantly smaller than those with vascular invasion, although lymphovascular invasion was observed even in the smallest tumor (0.3 cm) of this study. MCPyV DNA was present in 80% of 114 samples studied. Patients with MCPyV DNA-positive tumors had better overall survival than those with MCPyV DNA-negative tumors. There was a positive correlation with VEGFR-2 (n=21) and a negative correlation with endostatin (n=19) to tumor size, but no clear correlation to the development of metastasis.

Conclusions: MCC is a rare disease in Finland; its incidence rates are lower than in the USA but similar to those in the other Nordic countries. As in other studies, excision with clear margins and post-operative radiotherapy seems to decrease local recurrences. The finding of the high frequency of lymphovascular invasion supports the role of sentinel node biopsy even in the case of very small primary MCC tumors. MCPyV is frequently found in MCC and is associated with a more favorable outcome in these patients. The correlation between VEGFR-2 and endostatin with MCC tumor size supports the role of angiogenesis in MCC, but they have no prognostic relevance in the development of later metastases.

INTRODUCTION

Merkel cell carcinoma is a rare cutaneous malignancy that affects mostly elderly persons at an average age of 70 years at the time of diagnosis. Due to its rarity, there are only few epidemiological studies on MCC (Agelli et al. 2003, Hodgson 2005, Kaae et al. 2010, Riou-Gotta et al. 2009, Hussain et al. 2010, Holterhues et al. 2010). A typical Merkel cell carcinoma is a rapidly growing painless, purple nodule. It usually occurs on sun exposed skin areas, most commonly in the head and neck region. Exposure to ultraviolet radiation is a possible etiological factor, as well as immunosuppression. Recently, a small novel polyomavirus, named Merkel cell polyomavirus (MCPyV), was identified in Merkel cell carcinoma tumor tissue, suggesting that a viral infection might also be an etiological factor (Feng et al. 2008).

MCC has a high tendency for local recurrences (27–60%), regional lymph node metastases (45–75%), and distant metastases (18–52%). Several prognostic factors have been proposed for MCC, among them vascular invasion (Andea et al. 2008, Ng et al. 2008), but the most consistent predictor of survival in MCC to date is the presence or absence of lymph node metastases at the time of presentation (Bichakjian et al. 2007). Tumor growth and metastasizing requires the formation of new vessels, but data on angiogenic factors in Merkel cell carcinoma are scanty (Brunner et al. 2008)

Complete surgical excision with negative margins is the primary therapy for MCC, although the extent of surgery is under debate. Sentinel lymph node biopsy is recommended by many authors (Allen et al. 2005, NCCN 2010, Schwartz et al. 2011). Several studies support the use of postoperative radiation to minimize locoregional recurrence (Zhan et al. 2009, Rao et al. 2010, Pectasides et al. 2005, Bichakjian 2007). The effectiveness of chemotherapy is controversial, however (Henness et al. 2008).

REVIEW OF THE LITERATURE

1. NORMAL SKIN

The skin is composed of epidermis and dermis (Fig. 1). The outer skin layer, epidermis, is composed of several layers: keratin layer (stratum keratinosum), granular layer (stratum granulosum), spinous layer (stratum spinosum) and basal layer (stratum basale). The epidermis is avascular, nourished by diffusion from the dermis.

Keratinocytes are the major epidermal cell type, constituting 95% of the cells in the epidermis. They originate from cells in the basal layer and slowly migrate up toward the surface of the epidermis. Once the keratinocytes reach the skin surface, they are gradually shed and are replaced by younger cells pushed up from below. In between the keratinocytes, there are melanocytes, Langerhans cells and Merkel cells in the basal layer (McKee et al. 2005a).

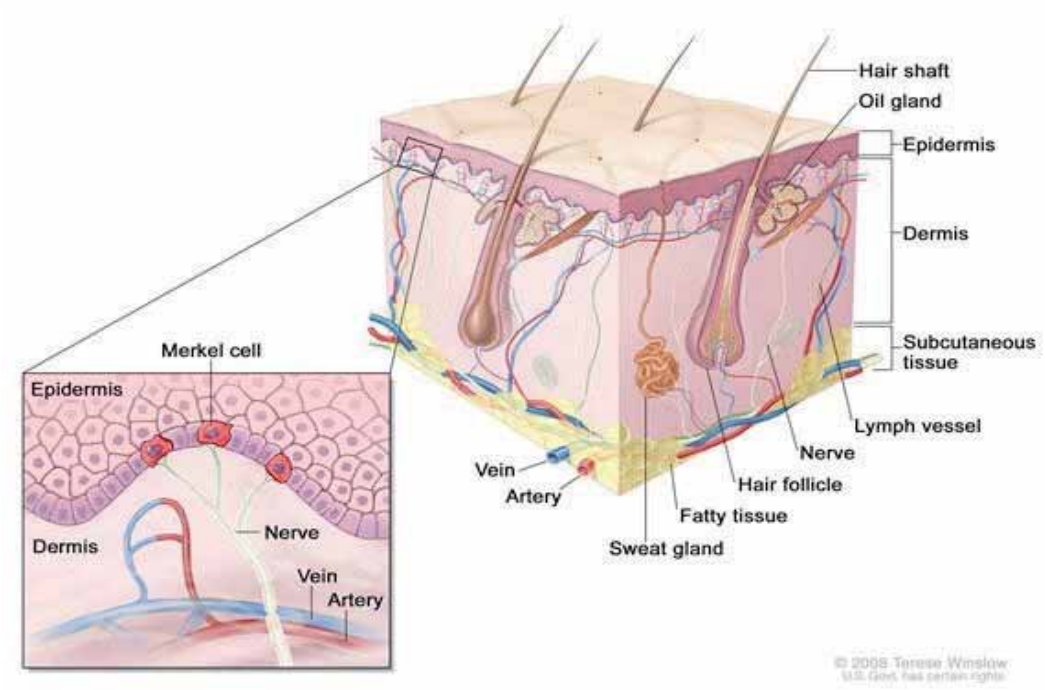


Figure 1. Normal skin structure (© Terese Winslow)

2. MERKEL CELL

Merkel cells, located in the basal layer of epidermis in certain distinct areas of mammalian hairy skin, were first described by Friedrich S.Merkel in 1875. He named these cells Tastzellen (touch cells) assuming that they had a sensory function within the skin due to their association with nerves. These Tastzellen came to be called "Merkel cells" after Tretjakoff designated them as such in 1902. Very recently, it has been demonstrated that Merkel cells play a role in light-touch responses and are an essential part of the somatosensory system (Marich et al. 2009).

Since the discovery of Merkel cells, the developmental origin of these cells has been debated. It has been speculated that Merkel cells are derived from neural crest, at least in quails and mice (Grim et al. 2000, Szeder et al. 2003). Another hypothesis suggests that Merkel cells originate from epidermal progenitors (Moll et al. 1996, Frigerio et al. 1983, Compton et al. 1990). A very recent study demonstrated that mammalian Merkel cells are descended from the epidermal lineage, and not from the neural crest lineage during embryonic development (Morrison et al. 2009). In adults, Merkel cells do not appear to divide under normal circumstances. Instead, they undergo slow turnover and are replaced by cells originating from epidermal stem cells, not through the proliferation of differentiated MCs (Van Keymeulen et al. 2009).

3. SKIN CANCERS

Skin cancers, as a group, are the most common form of cancer. There are different types of skin cancers depending on the cell of origin. Basalioma, basal cell carcinoma rises from the keratinocytes of the basal layer. It is the most common form of skin cancer, but it metastasizes extremely rarely (Menaker et al 2001). Squamous cell carcinoma (SCC) rises from keratinocytes of the spinous layer. It usually appears in sun-damaged skin with actinic keratosis and carcinoma in situ. It can also grow very rapidly and be invasive (Robinson 2001). Melanoma is a malignant tumor arising from normal melanocytes and it is more malignant than basalioma and SCC. It can begin either de novo from a melanocyte of normal skin or from a benign pigmented nevus (Langley et al. 2001).

Exposure to UV-radiation is the most important etiologic factor for all types of skin cancer (Mikkilineni et al. 2001). Also immunosuppression increases the risk of skin cancer (Teppo 1985, Koljonen 2009). The risk of skin cancer increases with advanced age, and the incidence of all skin cancers has increased along with a longer life-span, (Mikkilineni et al. 2001).

4. MERKEL CELL CARCINOMA

Merkel cell carcinoma (MCC) was first described in 1972, when Toker presented five cases of a trabecular cell carcinoma of the skin (Toker 1972). In 1978, Tang and Toker found dense-core granules typical of Merkel cells and other neuroendocrine cells in electron microscopical examination of these tumors, suggesting that the carcinoma had originated from the Merkel cells (Tang et al. 1978). Since then, a variety of names, e.g. neuroendocrine carcinoma of skin, cutaneous APUDoma, primary small cell carcinoma, have been used to describe this tumor. In the mid-1980s the name Merkel cell carcinoma was widely adopted.

It has been questioned whether MCC arises from normal Merkel cells. Firstly, normal Merkel cells are located in the epidermis, whereas most MCCs are confined solely to the dermis. In the literature there are case reports of 11 patients with MCC *in situ* or pagetoid MCC, in which MCC is confined exclusively to the epidermis (Smith et al. 1993, Brown et al. 2000, Ferringer et al. 2005). On the other hand, MCC shares some ultrastructural and immunohistochemical features with normal Merkel cells. It is currently thought that MCC is probably not derived from Merkel cells, but rather from a primitive pluripotent epidermal stem cells, which have the potential to differentiate along different cell lineages (Brown et al. 2009, Calder et al. 2010).

4.1. ETIOLOGY

4.1.1. Ultraviolet radiation

Clinical and epidemiologic evidence suggests that ultraviolet radiation plays a role in the pathogenesis of MCC. MCC usually occurs on sun-exposed skin areas with concomitant sun-related skin neoplasms i.e. actinic keratosis or squamous cell carcinoma. There is a correlation between the solar UV-B index (the estimated annual exposure to solar UV-B radiation) and the incidence of MCC (Miller et al. 1999). In a study by Lunder at al. (1998), the risk of MCC in psoriasis patients treated with PUVA (psoralen and UV-A) was calculated to be 100 times higher than in the general population. The same type of UV -induced mutations and chromosomal imbalancies are found MCC as in SCC (Popp et al. 2002).

UV radiation has a dual role in the development of skin cancers. The carcinogenic properties of ultraviolet light are mediated by its ability to generate DNA damage (Rünger et al. 2007), but it also diminishes cell-mediated immunity against malignant cells (Seiffert et al. 2002). MCC can occur also on non-sun-exposed areas of the body suggesting that there are also other factors contributing in the pathogenesis of MCC.

4.1.2. Immunosuppression

A body of evidence points to the association between immunosuppression and MCC. In an extensive review of 1024 MCC patients, 14.5% of these patients were receiving or had received immunosuppressive therapy (Medina-Franco et al. 2001). The incidence of MCC appears to be much higher (approximately 10-fold) in transplant recipients than in the general population (Penn et al. 1999, Buell et al. 2002, Koljonen et al. 2009, Lanoy et al. 2010). The rate of MCC after renal transplantation is calculated to be 0.13/ 1000 patient years (Bordea et al. 2004). These patients also tend to be younger at the time of diagnosis than the other MCC patients; half of them are under 50 years of age (Buell et al. 2002). The risk for Merkel cell carcinoma is also increased (approximately 13-fold) in people with HIV infection /AIDS indicating that immunosuppression is a risk factor for MCC (Engels et al. 2002, Lanoy et al. 2009).

4.1.3. Merkel cell polyomavirus

In 2008, a previously unknown polyomavirus was identified in tumor tissue from patients with MCC (Feng et al. 2008). This novel virus was designated Merkel cell polyomavirus (MCPyV). MCPyV belongs to the family of 5 human papillomaviruses, which are non-enveloped viruses with a circular double-stranded DNA genome of approximately 5 kb.

The first polyomaviruses, namely BKV and JCV, were discovered in 1971 (Gardner et al. 1971, Padgett et al. 1971). BKV is known to cause polyomavirus nephropathy in renal transplant patients and hemorrhagic cystitis in bone marrow transplant patients. JCV causes progressive multifocal leukoencephalopathy especially in HIV patients. It has been suggested that they are associated with certain human cancers, but so far there is no direct etiological evidence (Major et al. 1984, zur Hausen et al. 2008, Jiang et al. 2009). Primary infection with BKV and JCV is believed to occur in childhood and it is typically subclinical or manifests as a mild respiratory illness (Goudsmit et al. 1982, Monaco et al. 1998). After viral dissemination to the sites of life-long persistent infection, the virus enters a latent state. BKV is found in 30–50% of healthy kidney tissue and ureters and JCV, in addition to kidney epithelium, also in lymphocytes and oligodendrocytes. If an individual becomes immunocompromised (e.g. after a solid organ transplant, due to an autoimmune disease, or HIV) the virus is reactivated from its dormant state, leading to the above-mentioned severe diseases. Two other human papillomaviruses, KI and WU, are not yet linked to any known disease.

In the original discovery of MCPyV by Feng et al. (2008), DNA sequences were identified in 8 of 10 samples. Subsequent studies confirmed MCPyV to be present in 24–89% of MCC tumors (Becker et al. 2009, Foulongne et al. 2009, Andres et

al. 2010, Kassem et al. 2008, Duncavage et al. 2009). Interestingly, a study on patients from Australia and North America revealed that the proportion of tumors positive for MCPyV was much higher in North America (69%) than in Australia (24%) (Garneski et al. 2009).

Little is known about the natural history of MCPyV infection. MCPyV is found in normal skin and other tissues, but far lower amounts (Feng et al. 2008). Inflammatory monocytes have been proposed to be a reservoir for MCPyVs (Mertz et al. 2010). The virus is also found in other non-melanoma skin cancers, especially in immunocompromised individuals (Kassem et al. 2008). The prevalence of MCPyV antibody positivity correlates with age, increasing from 50% among children aged 15 years or younger to 80% among persons older than 50 years (Tolstov et al. 2009, Kean et al. 2009). These findings suggest that primary exposure to MCPyV, as to other polyomaviruses, is likely to occur already in childhood. The natural transmission route and site of initial infection have not yet been characterized for MCPyV.

Feng et al. (2008) found viral DNA to be integrated within the tumor genome in a clonal pattern. Also in one of the cases, DNA from the virus was integrated at the same site in the primary tumor as in a metastasis (Feng et al. 2008). These findings suggest that MCPyV infection and integration preceded clonal expansion of the tumor cells and metastasizing.

Several significant observations suggest that MCPyV contributes to carcinogenesis of MCC. It has been speculated whether MVCPyV is a prerequisite for the development of MCC, since the virus is not found in all MCC specimens (Garneski et al. 2008), but this could also be explained by different strains not detected by present PCR methods. A large majority of persons who become infected with MCV do not develop MCC; therefore other factors probably determine the risk for cancer (Tolstov et al. 2009).

4.2. MERKEL CELL CARCINOMA AND OTHER CANCERS

MCC is associated with a high incidence of other skin tumors and hematologic malignancies. Squamous cell carcinoma and basalioma are the most common second neoplasms with MCC (Brenner et al. 2001, Koljonen et al. 2010). One explanation for the high risk of other skin cancers in MCC is their common etiological factor, UV radiation.

Chronic lymphocytic leukemia is more than 30-fold overrepresented among patients with MCC (Heath et al. 2008). CLL is known to cause prolonged immune suppression, and some of the antineoplastic agents may further enhance the immunosuppression (Rashid et al. 2005). This association supports the role of immunosuppression in the pathogenesis of MCC. The association seems to be reciprocal; the incidence of CLL after MCC diagnosis is also increased (Koljonen et al. 2009).

A significantly elevated risk of subsequent cancer in MCC patients has been observed also for cancers of salivary gland, biliary sites other than liver and gallbladder, and non-Hodgkin lymphoma, multiple myeloma, and in men also brain cancer (Howard et al. 2006).

4.3. EPIDEMIOLOGY

Because MCC is a rare malignancy, few epidemiologic studies are available. Most of the epidemiologic data are from the Surveillance, Epidemiology, and End Results Program (SEER), which covers approximately 26% of the US population. According to SEER, the age-adjusted incidence rate was 0.24 / 100,000 person-years during 1986–1999 (Agelli 2003). In European studies, the incidence varies from 0.13–0.42 / 100,000 (Mills et al. 2006, Riou-Gotta et al. 2009, Hussain et al. 2010, Kaae et al. 2010). The rates are not directly comparable due to different age-adjustment methods. However, in all studies of MCC, the incidence is less than 1 per 100,000.

Another study based on SEER reported that the annual incidence has tripled from 0.15 to 0.44 / 100,000 during 1986–2001 (Hodgson 2005). There are possible explanations for the increased incidence: better diagnostic accuracy after immunohistochemistry, increased recognition of the disease, and a growing number of old people as well as people with immunosuppression.

Both sexes are affected with MCC. The ratio of men to women varies in different reports, but most studies report MCC to be more common in males (Agelli et al. 2003, Hodgson 2005, Heath et al. 2008, Allen et al. 2005, Jabbour et al. 2007, Medina-Franco et al. 2001, Andea et al. 2008). However, some studies have also reported a female predominance (Güler-Nizam et al. 2009, Ott et al. 1999, Llombart et al. 2005, Koljonen et al. 2003).

The incidence of MCC increases progressively with age. All studies invariably confirm that MCC is a disease of older people. Only 5% of MCC cases occur in patients under age 50 years of age, and it is extremely rare in childhood. The youngest known MCC patient was 7 years old (Schmid et al. 1992). The mean age at diagnosis in different studies ranges from 69–76 years (Alborees-Saavedra et al. 2010, Heath et al. 2008).

More than 90% of patients with MCC are Caucasian. According to an American study, the incidence rate among Caucasians was 11.3 times higher than in blacks, and 2.2 times higher than in all other ethnic groups (Agelli et al. 2003).

4.4. CLINICAL PRESENTATION

MCC is rarely suspected clinically at the time of diagnosis. In its early stage it can be misjudged by its appearance as a benign lesion, a cyst or abscess. A typical Merkel cell carcinoma is a rapidly growing painless, purple nodule (Fig. 2). Heath et al. proposed an acronym *AEIOU* (*A*symptomatic/lack of tenderness, *E*xpanding rapidly, *I*mmune suppression, *O*lder than 50 years, and *U*ltraviolet-exposed site on a person with fair skin) to describe the most significant symptoms of MCC (Heath et al. 2008). The most common location for MCC is the head and neck area (Albores-Saavedra et al. 2010, Heath et al. 2008, Agelli et al. 2003), followed by the extremities. On rare occasions it can occur in sun-protected areas, such as oral mucosa or the genital area.

The majority of patients present with a localized skin tumor and clinically negative nodes. Approximately 24–37% of patients have lymph node metastases and 6% have distant metastases at the time of diagnosis (Allen et al. 2005, Heath et al. 2008). MCC can infiltrate locally via dermal lymphatics, resulting in multiple satellite lesions.

A subset of patients present with metastases, usually lymph node metastasis, without a known primary MCC (Medina-Franco et al. 2001, Allen et al. 2005). Spontaneous regression of the primary tumor has been described (Junquera et al. 2005, Sais et al. 2002).

MCC has a high tendency for local recurrences (27–60%), regional lymph node metastases (45-75%), and distant metastases (18–52%).

The most common sites for distant metastases are lung (10–23%), brain (18%), bone (10–15%) and liver (13%). Other reported sites of distant metastases include testis, pancreas, heart, bone marrow, pleura, parotid, gastrointestinal tract, prostate, and bladder (Bichakjian et al. 2007).

The median time for a recurrence is 9 months (range 2–70 months); 91% of the recurrences occur within 2 years of diagnosis (Allen et al. 2005, Medina-Franco et al. 2001).

Figure 2. A rapidly grown Merkel cell carcinoma in the deltoid area of a 68-year old man. Two months earlier a small lump was excised at a primary health care center. The scar is visible in the middle of the tumor. The black spots are marks for sentinel node biopsy.

4.5. HISTOPATHOLOGICAL DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Under a light microscope, MCC appears typically as a dermal tumor nodule, which frequently extends into the subcutaneous fat. The classical histological features of MCC include sheets of small basophilic cells with scanty cytoplasm, fine evenly dispersed peppered chromatin, hyperchromatic nuclei, and small nucleoli (Fig. 3a). There are usually numerous mitotic figures, and apoptosis is often widespread (Sidhu et al. 2005, LeBoit et al. 2006). Tumor necrosis, perineural invasion and a high mitotic rate are common in MCC tumor samples. Vascular invasion is observed

in 38–60% of the samples in routine hematoxylin-eosin staining (Mott et al. 2004, Llombart et al. 2005, Feinmesser et al. 2004). Ulceration may be present, but is observed only in a minority of cases (Skelton et al. 1997, Mott et al. 2004). Epidermal involvement is rare, but there are cases in which MCC is limited to epidermis (MCC *in situ*) (Smith et al. 1993, Brown et al. 2000, Ferringer et al. 2005).

Three histological subtypes, with no clinical correlation, have been described. The most common subtype is an intermediate variant characterized by large, solid nodules and diffuse sheets of basophilic cells. The small cell variant is composed of small sized hyperchromatic cells resembling bronchial small cell carcinoma. The trabecular subtype is the least frequent, and consists of ribbons of small basophilic cells separated by connective tissue (Gould et al. 1985, Sidhu et al. 2005).

In addition to the typical histological appearance in hematoxylin-eosin staining, the diagnosis of MCC is confirmed if an appropriate immunohistochemical profile is identified (Table 1). Typically, MCC expresses both neuroendocrine (chromogranin A, synaptophysin, neuron specific enolase) and cytokeratin markers (cytokeratin 20, CAM 5.2). However, it is negative for S100 and leukocyte common antigens (LCA) and thyroid transcriptase factor-1 (TTF-1), distinguishing it from melanoma, lymphoma and pulmonary small cell carcinoma, respectively (McKee et al. 2005b, LeBoit et al. 2006).

Cytokeratin 20 is expressed in benign and malignant digestive tract epithelium, gastrointestinal adenocarcinomas, urothelium, Merkel cells, and MCC (Moll et al. 1992). The cytokeratin positivity in MCC predominantly manifests itself as a distinct perinuclear dot-like pattern (Fig. 3b), a feature distinguishing it from small cell carcinomas of internal organs (Sidhu et al. 2005). In earlier years, electron microscopy was useful in diagnostics to document neuroendocrine features, but today immunohistochemical markers have replaced it.

Table 1. Differential diagnosis of Merkel cell carcinoma by immunohistochemistry (Modified from McKee et al. 2005b, Llombart et al. 2005, Hanly et al. 2000, Koljonen et al. 2005, Goessling et al. 2002, Poulsen et al. 2004, Bichakjian et al. 2007, Mott et al. 2004).

	CK-20 (%)	TTF-1 (%)	LCA (%)	S-100 (%)	NFP (%)	NSE (%)	SYP (%)	ChrA (%)	NCAM
MCC	89-100	0	0	0	50-100	80	95	80-100	100
SCLC	3-33	83-100	0	0	0	64			
Lymph	0		98	6	0	11			
SCM	0	0	0	97	0	71			
BCC	0								

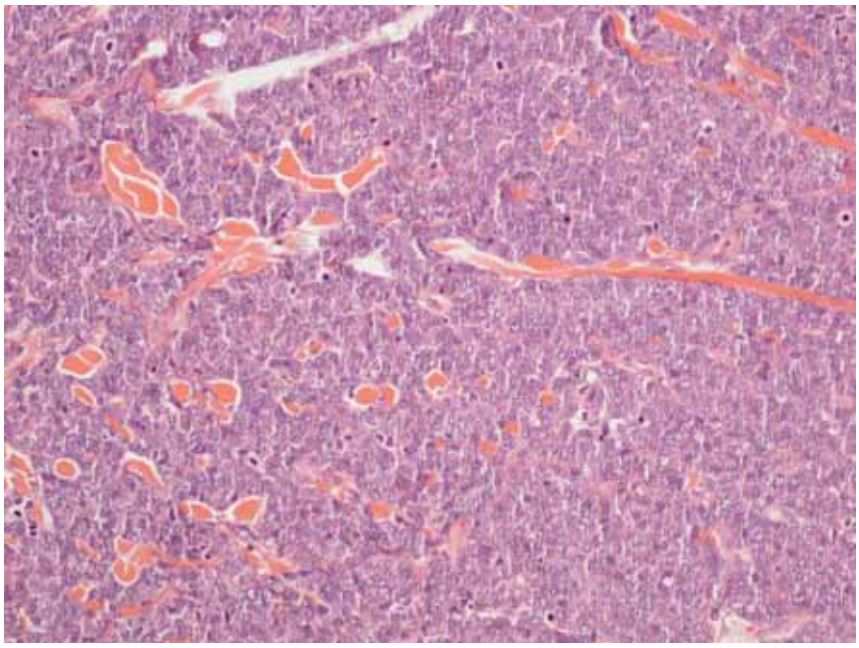


Figure 3a. MCC in HE staining. Original magnification x200.

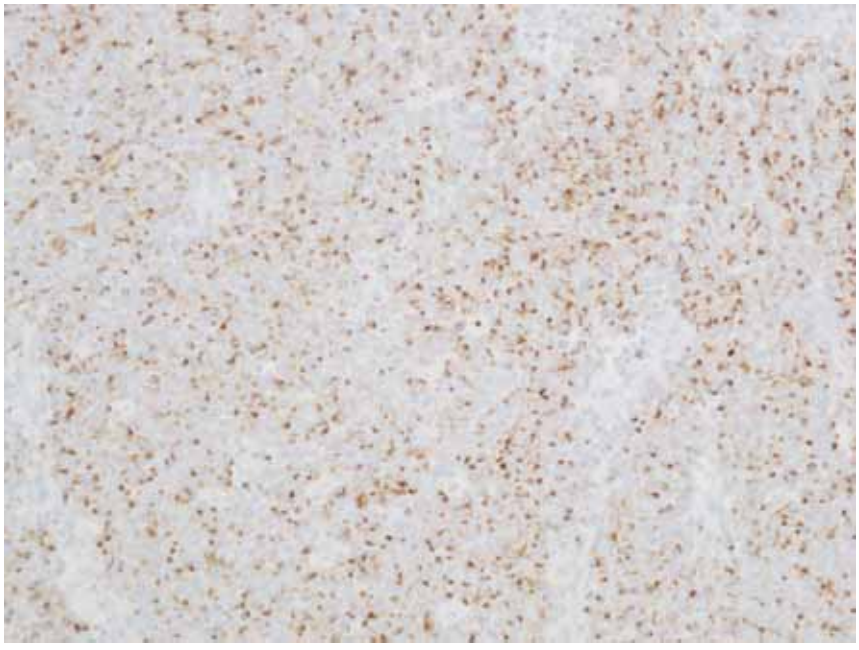


Figure 3b. Positive CK-20 staining. Original magnification x200.

4.6. STAGING AND PROGNOSIS

The prognosis of MCC has been considered rather poor. The overall survival rates are reported to be 30–75%. The most consistent predictor of survival in MCC to date is the presence or absence of lymph node metastases at the time of presentation (Bichakjian et al. 2007). Tumor size as a prognostic factor is also reflected in survival rates. There are a few staging systems in use, but unfortunately they are quite inconsistent with each other. A 3-tiered staging system was proposed by Yienpruksawan in 1991 based on the presence or absence of lymph node or distant metastases (Yienpruksawan et al. 1991). Later, Stage I was divided into two, according to tumor size as it was found to be an independent factor predicting survival.

The Memorial Sloan-Kettering Cancer Center (MSKCC) classification has been the most commonly used staging system (Table 2). In this classification the patients are grouped into four stages: local disease with primary tumor < 2cm or ≥ 2cm (Stages I and II), lymph-node positive (Stage III) or distant metastatic disease (Stage IV) (Allen et al. 1999, Koljonen et al. 2003). This staging system was used in our studies.

Table 2. Memorial Sloan-Kettering Cancer Center staging system (Allen et al. 2005)

Stage	MCC	5-year disease specific survival (%)
I	Local disease <2cm	81
II	Local disease ≥2cm	67
III	Regional nodal disease	52
IV	Distant metastatic disease	11

For historical reasons, this staging system does not differentiate whether nodal staging is performed only clinically, or also histopathologically. However, when histopathological nodal staging is used, a subgroup of patients with an excellent prognosis can be identified: the survival of patients with histopathologically negative nodes was 97% compared to 52% in pathologically nodal positive patients (Allen et al. 2005, Lemos et al. 2010). Therefore, the American Joint Committee on Cancer (AJCC) has very recently developed a new MCC-specific consensus staging system (Table 3). Patients with primary Merkel cell carcinoma and no evidence of regional or distant metastases are divided into two stages: Stage I for primary tumors no more than 2 cm in size, and Stage II for primary tumors larger than 2 cm in size. Stages I and II are divided further into substages A and B, based on the method of nodal evaluation. Patients who have a histopathologically verified node-negative disease in sentinel node biopsy (pN0) have a better survival rate (substaged as A) than those who are evaluated only clinically (cN0)(substaged as B). Stage II has

an additional substage (IIC) for tumors that display extracutaneous invasion (T4), and that have a negative node status regardless of whether the negative node status was established microscopically or clinically. Stage III is also divided into A and B categories, i.e. for patients with a microscopically positive finding (N1a)(IIIA), and those with clinically detected lymph node metastasis (N1b)(IIIB). There are no subgroups of stage IV MCC (AJCC 2010).

Table 3. American Joint Committee on Cancer consensus staging system for MCC (AJCC 2010)

Primary tumor (T)

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor (e.g. nodal/metastatic presentation without associated primary tumor)
Tis	*In situ* primary tumor
T1	≤2 cm maximum tumor dimension
T2	>2 cm but ≤5 cm maximum tumor dimension
T3	>5 cm maximum tumor dimension
T4	Primary tumor invades bone, muscle, fascia or cartilage

Regional lymph nodes (N)

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
cN0	Nodes negative in clinical examination (no histopathological examination performed)
pN0	Nodes negative by histopathological examination
N1	Metastasis in regional lymph node(s)
N1a	Micrometastasis (diagnosed after sentinel or elective lymphadenectomy)
N1b	Macrometastasis (clinically detectable nodal metastases confirmed by CLND or needle biopsy)
N2	In transit metastasis

Distant metastases (M)

M0	No distant metastasis
M1	Metastasis beyond regional lymph nodes
M1a	Metastasis to skin, subcutaneous tissues or distant lymph nodes
M1b	Metastasis to lung
M1c	Metastasis to all other visceral sites

Anatomic stage / prognostic groups

Stage 0	Tis	N0	M0
Stage IA	T1	pN0	M0
Stage IB	T1	cN0	M0
Stage IIA	T2/T3	pN0	M0
Stage IIB	T2/T3	cN0	M0
Stage IIC	T4	N0	M0
Stage IIIA	Any T	N1a	M0
Stage IIIB	Any T	N1b/N2	M0
Stage IV	Any T	Any N	M1

4.7. TREATMENT

As MCC prognosis is highly associated with the extent of the disease at presentation, early detection and prompt referral to a clinic with adequate facilities is the cornerstone of successful treatment (Allen et. al 2005, Heath et al. 2008). As in the case of other cancers, treatment varies based on the tumor stage (NCCN 2010). Staging is therefore an essential component of patient management. Due to its rarity and aggressive behavior, a multidisciplinary approach is recommended. Surgery is the mainstay of treatment, but it can result in significant disfigurement and impaired function, and therefore requires careful planning of the incision and reconstruction (Gonzales et al. 2010).

4.7.1. Localized disease (Stages I and II)

Surgical excision with negative margins is the mainstay of primary treatment of MCC, but the optimal surgical margin is still under debate. In the older series, margins ≥3cm were associated with a reduction in local recurrences (Yiengpruksawan et al. 1991, O'Connor et al. 1996) and therefore wide local excision with 2–3 cm surgical margins has been historically recommended. Later, Ott et al. (1999) reported no difference in outcome with margins of 2 cm or more compared to margins less than 2cm. Neither did Gillenwater et al. (2001) find any difference regardless of whether the margins were under 1 cm, 1–2 cm, or more than 2 cm, but this might be explained by the small study population of 18 patients in their study. The same observation was confirmed in a much larger study of 251 patients by Allen et al. (2005) demonstrating that surgical clear margins < 1 cm did not lead to higher recurrence rates than did margins ≥ 1 cm. Also Poulsen et al. (2010) found no

additional benefit of a wide surgical excision (>2cm) of the primary lesion in their series of 60 patients, although the authors assumed that this might have been due to the high use of adjuvant radiotherapy. Low local recurrence rates have been reported with even smaller margins. Bajetta et al. (2009) stated in their study of 95 patients that margin-negative excision was sufficient to decrease local recurrence and improve survival.

Mohs micrographic surgery, first described by Dr. Mohs in 1941, is a specialized technique that combines pathology with surgery and provides complete assessment of the margins intraoperatively (Mohs et al. 1941, Minton 2008).

As controlled trials comparing different margins of excision have not yet been performed, controversies do exist. However, there is strong agreement that clear surgical margins are the goal of surgical treatment. In an extensive review, Bichakjian et al. (2007) concluded, based on current evidence, that margins of 1 cm will frequently be negative for small lesions that measure <2 cm in greatest dimension, and that 2 cm margins should be reserved for larger lesions >2 cm.

Disagreement exists on whether excision of the primary MCC should be followed by adjuvant radiotherapy to the tumor bed. MCC is known to be radiosensitive (Leonard et el. 1995) and many studies support the use of postoperative radiation to improve the outcome in MCC (Morrison et el. 1990, Meeuwissen et al. 1995, Poulsen et el. 2009, Lewis et el. 2006, Warner et el. 2008, Senchenkov et el. 2007), although contradictory reports exist (Medina-Franco et al. 2001, Veness et al. 2005, Allen et al. 2005).

In some studies, local and regional recurrences are grouped together as locoregional recurrences, thus overestimating the frequency of true local recurrences. In the absence of local recurrence, lymph node recurrence usually represents the delayed manifestation of micrometastatic disease present at the time of treatment of the primary tumor (misclassification error, occult Stage III disease) rather than the result of inadequate local therapy (Bichakjian et al. 2007).

Some authors recommend adjuvant radiation therapy to all MCC patients (Zhan et al. 2009, Rao et al. 2010, Pectasides et al. 2005), while others recommend it only when clear margins cannot be obtained, or the tumor is larger than 1.5–2 cm, adverse risk factors are present, or sentinel node biopsy is found positive (Bichakjian et al. 2007).

There are reports of treating inoperable patients with localized MCC by radiotherapy only; the outcomes have been comparable to those obtained with surgery and radiotherapy (Mortier et al. 2003). However, radiation as a monotherapy should be reserved only for those patients who cannot tolerate surgical treatment of the primary tumor.

4.7.1.a Sentinel lymph node biopsy

Sentinel lymph node is the first lymph node(s) draining the tumor site, and it is therefore first reached by metastasizing tumor cells. The concept of sentinel lymph node biopsy (SNB) is based on the hypothesis that if the sentinel node does not harbor metastatic cells, it is highly unlikely that cancer cells have spread to any other part of the body. The sentinel node can be identified by using lymphoscintigraphy and intraoperative mapping of the lymphatics. SNB was first introduced by Cabanas in 1977 for treating penile carcinoma (Cabanas 1977). Later, it has become routine for breast cancer and melanoma staging and treatment protocols. Sentinel node biopsy has proved reliable also in MCC (Pfeifer et al. 1997, Messina et al. 1997, Hill et al. 1999, Wasserberg et al. 2000, Mehrany et al. 2002).

It is estimated that 25–33% of the patients with clinically negative nodes do have microscopic metastases (Allen et al. 2005, Gupta et al. 2006). Stokes et al. (2009) have found that patients with MCC ≤1 cm are unlikely to have regional lymph node metastases), but other studies have shown that the incidence of occult nodal disease is not associated with tumor size (Allen et al. 2005, Sarnaik et al. 2009). Histopathological nodal evaluation has been shown to improve prognostic accuracy in MCC (Allen et al. 2005, Lemos et al. 2010), and it can be used to identify a group of patients with excellent long-term survival (Allen et al. 2005). Therefore SNB is recommended for all MCC patients with localized disease (NCCN 2010, Allen et al. 2005, Schwartz et al. 2011).

4.7.2. Regional disease (Stage III)

The currently recommended practice for a sentinel lymph node positive disease is completion lymph node dissection (CLND), if this can be performed safely. The advantage of CLND is that it enables full assessment of positive nodes, but there is a risk of postoperative lymphedema. If the morbidity of CLND is regarded unacceptable, or sentinel node biopsy is positive only by immunohistochemical methods (not detected in HE-staining), an alternative therapy might be radiotherapy to the lymph node basin (NCCN 2010). If there is extensive lymph node disease or extracapsular lymph node extension, adjuvant radiotherapy should be considered.

If the patient has palpable lymph nodes at the time of diagnosis, an ultrasound guided fine needle biopsy or core biopsy should be performed, followed by CLND if metastasis is confirmed. However, very recently lymph node irradiation was shown to give an excellent control rate comparable to CLND, for both microscopic and palpable lymph node disease (Fang et al. 2010). Considering the rarity of MCC and the lack of randomized controlled prospective trials, there are no definitive treatment guidelines with full consensus, and therefore each patient should undergo a multidisciplinary tumor board consultation.

4.7.3. Distant metastatic disease (Stage IV)

When a patient is found to have distant metastases at the time of diagnosis, the role of surgery and radiation is mostly palliative. Several chemotherapeutic agents have been used with no convincing results (Tai et al. 2000, Henness et al. 2008). Also the poor performance status of many elderly MCC patients with advanced disease diminishes the use of the toxic regimen.

5. TUMOR BIOLOGY

5.1. ANGIOGENESIS IN TUMOR GROWTH AND METASTASES

Angiogenesis, i.e. the formation of new blood vessels by sprouting or splitting from established blood vessels, is essential for both normal development and homeostasis (female reproductive cycle, tissue repair and wound healing). It also occurs in certain pathological conditions (cancer, macular degeneration, diabetic retinopathy) (Veikkola et al. 1999, Klagsbrun et al. 1996). It is characterized by a cascade of events, including enzymatic degradation of basement membrane, endothelial cell migration and proliferation, and tube formation (Klagsbrun et al. 1996). Under physiological conditions, angiogenesis is regulated by the local balance of the endogenous stimulators and inhibitors of this process.

The hypothesis that tumor growth depends on angiogenesis was first proposed by Judah Folkman in 1971 (Folkman 1971). Induction of angiogenesis is required for tumors to grow beyond 1–2 mm in diameter, which is the limit of simple diffusion of nutrients and oxygen (Folkman 1990). This angiogenic switch is caused by an imbalance between pro- and anti-angiogenic factors. It can occur at different stages of the tumor-progression pathway, depending on the tumor type and the environment (Bergers et al. 2003). Angiogenesis is also needed to enable the tumor to progress to metastatic disease. In order to develop metastases, several steps must be completed successfully. Cells from the primary tumor need to intravasate to the body's circulatory system and survive in the circulation until they become arrested at a new site. The tumor cells can also reach blood circulation via efferent lymphatic vessels and the venous system. Several factors, including tumor type and organ environment as well as vascular flow pattern, influence where the metastatic process of each cancer type will preferably start. After their arrest in a specific organ,

the tumor cells must extravasate into the surrounding tissue and initiate growth. Finally they have to vascularize to develop into anything more than a dormant pre-angiogenic micrometastasis (Chambers et al. 2002).

5.1.1. Vascular endothelial growth factor

VEGFs and their receptors are prime regulators of both physiological and pathological angiogenesis. VEGFs are a family of secreted dimeric glycoproteins. To date, there are 5 known members in this family in mammals: VEGF, placental growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D. They all have at least some angiogenic potential, although VEGF-C and VEGF-D mostly function as lymphangiogenic growth factors (Lohela et al. 2009).

VEGF (also known as VEGF-A) is a mitogen that is specific to endothelial cells (Leung et al. 1989, Keck et al. 1989). It promotes the growth of vascular endothelial cells *in vitro*, and induces a potent angiogenic response *in vivo*. It also maintains survival of endothelial cells by preventing apoptosis. In addition, VEGF functions as a regulator of vascular permeability and is able to induce vascular leakage, which is considered important for the initiation of angiogenesis (Veikkola et al. 1999, Ferrara et al. 2003).

VEGF is produced by numerous cell types, including tumor cells, macrophages, T cells, smooth muscle cells, kidney cells, mesangial cells, keratinocytes, astrocytes and osteoblasts (Klagsbrun et al. 1996).

Hypoxia is considered to be the major regulator of VEGF expression, but there is a variety of growth factors and cytokines that act by up-regulating VEGF expression (Klagsbrun et al. 1996). Specific transforming events also result in the induction of VEGF gene expression. Oncogenic mutations or amplification of Ras lead to VEGF up-regulation (Ferrara et al. 2003).

5.1.2. Vascular endothelial growth factor receptors

VEGF has two transmembrane receptor tyrosine kinases, VEGFR-1 (Flt-1, fms-like tyrosine kinase) and VEGFR-2 (Flk-1/ KDR, fetal liver kinase-1/ kinase domain region) (Terman et al. 1991, DeVries et al.1992). They are primarily located on endothelial cell surface, but found on some non-vascular cells as well (Katoh et al. 1995, Kim et al. 1999, Holmes et al. 2007). VEGF binds with high affinity to these two receptors, but VEGFR-2 is the major signaling receptor mediating most of VEGF's biological activity in endothelial cells (Holmes et al. 2007). Also VEGFR-3 belongs to the family of VEGFRs; it is expressed mainly in the lymphatic endothelium

and serves as a receptor for the lymphovascular growth factors VEGF-C and -D (Kaipainen et al. 1995, Joukov et al. 1996, Achen et al. 1998).

5.1.3. Endogenous angioinhibitors

Endogenous inhibitors of angiogenesis are defined as proteins or fragments of proteins that are formed in the body and that can inhibit the formation of blood vessels. At least 27 different anti-angiogenic proteins and small molecules are known to exist (Nyberg et al. 2005, Ribatti 2009).

Angiostatin was the first angioinhibitor discovered. Angiostatin specifically inhibits the proliferation of growing endothelial cells, but it has no effect on resting confluent endothelial cells or on other cell types (O'Reilly et al. 1994).

Endostatin was discovered in 1997 (O'Reilly et al. 1997), and it is the most studied of the endogenous angioinhibitors. It is a 20-kDa carboxyl-terminal fragment of collagen XVIII, a basement membrane protein that can be generated by elastase and certain other proteases. The distribution of endostatin is widespread in normal vessel walls, e.g. in the skin (Miosge et al. 1999), and it can be detected in blood circulation. The ability of endostatin to inhibit tumor growth and angiogenesis in vivo has been demonstrated in several studies performed on animal models (O'Reilly et al. 1997, Blezinger et al. 1999, Ramchandran et al. 2002, Sund et al. 2005).

5.1.4. Anti-angiogenic therapy

The fact that tumor growth is angiogenesis-dependent has led to extensive research on anti-angiogenic therapeutic options based on the notion that blocking angiogenesis could be a strategy for arresting tumor growth and restricting further development of metastasis. Targeting endothelial cells rather than the tumor cells themselves is promising, since endothelial cells are genetically stable and therefore less prone to accumulate mutations leading to drug resistance (Boehm et al. 1997). Abnormal angiogenesis can be prevented by several mechanisms: anti-VEGF antibodies, VEGFR blocking agents, or with a soluble extracellular component of VEGFR that binds soluble VEGF (so-called VEGF Trap) (Holash et al. 2002). The use of anti-VEGF monoclonal antibody bevacizumab has been approved for treating patients with metastatic colorectal carcinoma, non-small cell carcinoma and metastatic breast carcinoma in combination with chemotherapy (Hurwitz et al. 2004, Miller et al. 2007, Sandler et al. 2006). VEGFR tyrosine kinase inhibitors sorafenib and sunitinib have been shown to mediate an anti-angiogenic effect on patients with renal cell carcinoma and hepatocellular carcinoma (Escudier et al.

2007, Llovet et al. 2008). A recently introduced recombinant human endostatin has been found to exert anti-angiogenic effects via similar mechanisms as endogenous endostatin (Ling et al. 2007).

5.2. TUMOR LYMPHATIC VESSELS

Lymphatic vessels are essential for maintaining fluid balance in the body by collecting interstitial fluid from peripheral tissues and returning it to the blood circulation. Lymphatics are also important in the absorption of dietary fat and for immune functions, as well as for the metastatic spread of cancer (Cueni et al. 2008, Sleeman et al. 2009).

The initial step of a tumor escaping beyond its boundaries is often histologically seen as invasion of local lymphatic vessels. Lymphovascular invasion has been shown to be indicative of an unfavorable prognosis and the presence of lymph node metastasis is the major prognostic factor for many cancers underlining the import role of the lymphatic structures in the progression of the disease (Das et al. 2008).

Previously it was thought that lymphatic metastasis was a passive process in which detached tumor cells enter pre-existing lymphatic vessels and then get trapped into the lymph nodes (Pepper et al. 2001). However, it is now widely accepted that embolic dissemination of tumor cells within the newly formed lymphatic vessels also accounts for lymph node metastases (Cueni et al. 2008, Sleeman et al. 2009). It has been demonstrated that tumors can actively induce lymphangiogenesis to increase the lymphatic density within or near the tumor. This in turn increases the probability of tumor cells to invade into the lymphatic system, although the exact mechanism of invasion is less understood (Raica et al. 2010). In both experimental and human tumors, the expression of lymphangiogenic growth factors VEGF-C and/or VEGF-D correlate with vascular invasion, lymph vessel and lymph node involvement, distant metastases and in some cases, poor clinical outcome (Achen et al. 2008).

The importance of lymphangiogenesis may vary depending on the tumor type and location. In some animal models metastatic spread to lymph nodes occurred also in the absence of tumor lymphangiogenesis, presumably via pre-existing lymphatic vessels (Wong et al. 2005). Also chemokines secreted by lymphatic vessels are believed to play a role in attracting tumor cells and facilitating the entry of tumor cells into the lymphatic system (Sleeman et al. 2009, Shields et al. 2007).

Several endothelial markers are used to identify blood vessels and lymphatic vessels. CD-31, also known as platelet endothelial cell adhesion molecule 1 (PECAM1), is a type I integral membrane glycoprotein and a member of the immunoglobulin superfamily of cell surface receptors. It is found predominantly on the surface of endothelial cells. It is a panvascular endothelial marker expressed in both lymphatic

and blood endothelial cells, but it is less pronounced in the former (Podgrabinska et al. 2002). D2-40 is a monoclonal antibody directed against human podoplanin, a transmembrane mucoprotein expressed in lymphatic endothelial cells. It is a sensitive and specific marker of lymphatic endothelium, and does not react with blood vessel endothelium (Kahn et al. 2002a, Kahn et al. 2002b). Other markers for distinguishing lymphatic and vascular endothelium include VEGFR-3 (the tyrosine kinase receptor for VEGF-C and –D), prox-1 (the homeobox gene product that is involved in the developmental regulation of the lymphatic system), and LYVE-1 (a hyaluronan receptor selectively expressed in lymphatic vessels) (Sleeman et al. 2001, Raica et al. 2010).

AIMS OF THE STUDY

I	To explore the epidemiology of MCC, its incidence, clinical picture and survival in the Finnish population
II	To investigate (lympho)vascular invasion and its prognostic significance
III	To examine the clinical features of Merkel cell polyoma virus -related Merkel cell carcinomas
IV-V	To study VEGRF-2 and endostatin expression and their value as a prognostic factors

PATIENTS AND METHODS

Data were obtained from the Finnish Cancer Registry on all cases with a notation of Merkel cell carcinoma or small cell carcinoma of the skin during 1979–2008; altogether 295 cases were reviewed. The first notation of MCC is for a tissue sample from 1979. This sample was originally interpreted as a reticular cell carcinoma or anaplastic carcinoma. The correct diagnosis of MCC was made retrospectively 18 years later when this patient developed B-cell lymphoma and the archival tissue sample from 1979 was re-evaluated. The diagnosis of MCC was added to the registry later. The first diagnosis of MCC in Finland was made in 1983.

For the clinical series, formalin-fixed paraffin-embedded tissue samples of patients with MCC during 1979–2004 (n=207) were tracked and collected, if available. The original tissue sample (biopsy, first excision, re-excision or metastasis) was available from 193 patients. The diagnoses were confirmed in a blinded fashion by two of the researchers (TB and HK).

For histopathological confirmation of the diagnosis, we required that the tumor morphology was consistent with MCC in the hematoxylin-eosin -stained tissue sections, and that these were positive by immunostaining for cytokeratin 20 (CK-20). If CK-20 was negative, but morphology consistent with MCC, the diagnosis was confirmed by using immunostaining for synaptophysin and chromogranin A. Negative immunostaining for thyroid transcriptase factor 1 (TTF-1) was also required to exclude metastatic small cell lung carcinoma. In 12 patients the diagnosis of MCC could not be confirmed in the re-evaluation (10 cases were inconsistent with MCC morphology and 2 cases were TTF-1 positive). The remaining 181 patients were included in the epidemiologic-clinical study (Study I) and were reviewed in more detail for clinical course and treatment. Tumor size (the greatest surface dimension) was measured from the HE-stained slides whenever feasible. Otherwise we used the diameter that was reported in the case records.

Some of the tissue samples were further analyzed for vascular invasion (n=126) by immunohistochemistry, using vascular endothelial markers CD-31 and D2-40, and for MCPyV (n=114) by using polymerase chain reaction (PCR) and quantitative PCR. Immunohistochemical analyses for the expression of VEGFR-2 (n=21) and endostatin (n=19) were performed. The studies were conducted at different phases of material collection, and therefore the number of patients and samples are not identical.

EPIDEMIOLOGICAL- CLINICAL STUDY (STUDY I)

The incidence (adjusted to the world standard population) of MCC in Finland during 1979–2008 was calculated.

For the clinical series, 181 patients were included in this study (Tables 4 and 5). More detailed data for clinical course and treatment were obtained from the hospital records and health care centers throughout the country.

The minimum excision margin (minimum distance of healthy tissue from tumor to resection margin, either lateral or deep) was also measured in the histological samples and the minimum cumulative excision margin in the case of re-excisions was calculated.

Intralesional excision denotes cases in which tumor cells are observed at the resection margin on histological examination, or the free margin is less than 1 mm, or only biopsy was performed. The marginal excision group consists of cases in which the distance from the tumor to the resection margin measures 0.1–1.9 cm. Wide excision means that the resection margin was least 2 cm.

Relative survival ratio (RSR) was calculated for these 181 patients. RSR describes excess mortality due to MCC, and closely resembles disease-specific survival. It was calculated from the date of histological diagnosis to the date of death or to the closing date of that analysis (December 31, 2007).

Table 4. Patient and tumor characteristics of 181 patients (Study I)

	Value	No. of patients	%
Sex			
female		125	69.1
male		56	30.9
Age (years)			
mean	75.9		
median	78		
range	27-100		
0–50		6	3.3
51–74		58	32.1
75–100		117	64.6
Tumor location			
head and neck		102	53.3
trunk		22	12.2
upper extremity		27	14.9
lower extremity		22	12.2
primary unknown		8	4.4
Tumor size (cm)			
mean	1.78		
median	1.4		
range	0.3–8.5		

Table 5. Stage at presentation and tumor size by sex

	Females (n=125)	Males (n=56)	All (n=181)
Stage I	80 (64%)	28 (50%)	108
tumor size (mean, mm)	10.8	9.9	10.6
Stage II	32 (26%)	15 (27%)	47
tumor size (mean, mm)	28.8	34.7	30.7
Stage III	10 (8%)	8 (14%)	18
tumor size (mean, mm)	32.4	31.0	31.8
Stage IV	3 (2%)	5 (9%)	8
tumor size (mean, mm)	24.7	13.0	18.8

(LYMPHO)VASCULAR INVASION (STUDY II)

Of the 181 patients described in Study I, those with a known primary tumor, sufficient clinical data at the time of the study, and a tissue sample of the primary tumor of adequate quality were included in this study (n=126) (Table 6).

To detect vascular structures, immunohistochemical staining was performed using antibodies for CD-31 and D2-40. The protocol for preparing tissue sections, immunohistochemistry and statistical analysis are described in detail in Study II.

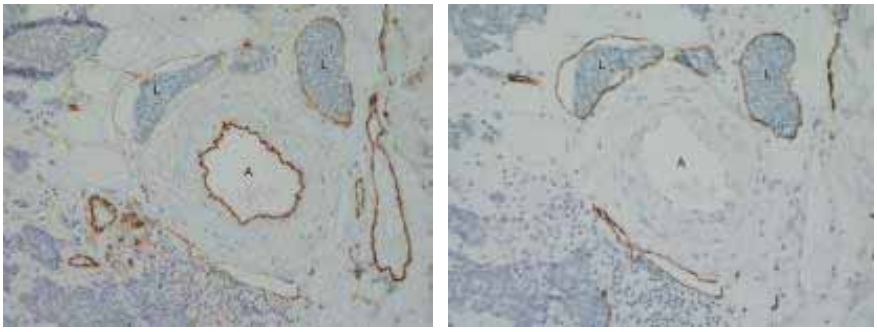
One slide of each sample was examined under a light microscope by two researchers (TB and HK) blinded to the clinical data and disease outcome.

The observations were defined by the following terms:

Vascular invasion – a cluster of tumor cells within a vascular lumen, identified by CD31 or D2-40 staining

Lymphovascular invasion – a tumor embolus within a D2-40 positive lining (Fig. 4)

Blood vascular invasion – a tumor embolus within a CD31 positive structure that was negative in D2-40 staining (Fig. 5).



a b

Figure 4. Lymphovascular invasion. Staining with CD31 (a) and D2-40 (b). A cluster of tumor cells within a lymph vessel (L). Endothelial cells of an artery (A) stained with CD31, but remained negative with D2-40 staining. Original magnification x200.

a b

Figure 5. Blood vascular invasion. Staining with CD31 (a), and D2-40 (b). A cluster of tumor cells within a blood vessel (B) identified by CD31 staining. The endothelial cells remained negative in D2-40 staining. Original magnification x200.

Table 6. Clinical characteristics of Merkel cell carcinoma patients at diagnosis (Study II)

	No of patients (n=126)	%
Sex		
female	90	71.4
male	36	28.6
Age (years)		
mean	77.6	
median	79	
range	50–100	
Tumor location		
head or neck	73	57.9
upper extremities	21	16.7
trunk	14	11.1
lower extremities	18	14.3
Tumor size (cm)		
mean	1.86	
median	1.5	
range	0.3–8.5	
Stage at diagnosis		
I Local only <2cm	75	59.5
II Local only ≥2cm	36	28.6
III Nodal*	11	8.7
IV Distant metastatic	4	3.2

*nodal disease assessed by palpation or histological evaluation (when performed)

MERKEL CELL POLYOMA VIRUS (STUDY III)

Of the 207 subjects identified from the Finnish Cancer Registry, 93 were excluded from this study for the following reasons: no formalin-fixed paraffin-embedded archival tumor tissue of the primary tumor was available for review; the diagnosis of Merkel cell carcinoma could not be confirmed; the site of the primary tumor was not known; sufficient clinical information was not available; DNA of adequate quality could not be obtained from the archival tumor tissue; or the quantitative polymerase chain reaction assay we used to detect MCPyV DNA was not informative. The remaining 114 subjects with confirmed Merkel cell carcinoma and with adequate tumor tissue DNA and clinical information available were included in this study (Table 7).

As control samples, we obtained 22 formalin-fixed paraffin-embedded tissue samples from the archives of the Department of Pathology (Helsinki University Central Hospital) comprising glioblastoma (n = 8), skin melanoma (n = 7), or histologically normal tissue (n = 7; one each of skin, stomach, colon, skeletal muscle, and lymph node, and two bone marrow samples); samples within each category were selected at random.

Detection of MCPyV DNA and statistical analysis are described in detail in Study III.

Table 7. Patient and tumor characteristics of 114 patients (Study III)

		No. of patients	%
Sex			
female		80	70.2
male		34	29.8
Age (years)			
median	78		
range	35–100		
Tumor location			
head and neck		58	50.9
trunk		17	14.9
limb		39	34.2
Tumor size (cm)			
median	1.6		
range	0.3–8.5		
Stage at presentation			
I		61	53.5
II		39	34.2
III		10	8.8
IV		4	3.5

VEGFR-2 AND ENDOSTATIN (STUDIES IV-V)

These studies comprised 21 patients treated for MCC between 1987 and 2003 at the Department of Plastic Surgery, Helsinki University Central Hospital, Finland (Table 8). The material was limited because the studies were conducted before all the data from the Finnish Cancer Registry were available. The diagnoses were confirmed by the methods described above. Tumor size (the greatest surface dimension) was measured from hematoxylin-eosin -stained slides and documented as <2 cm or ≥2 cm; this cutoff point was chosen based on staging.

Table 8. Patient and tumor characteristics of VEGFR-2 and the endostatin study (Studies IV-V)

	VEGFR-2 study (n=21)	Endostatin study (n=19)
Sex (number of patients)		
female	12	10
male	9	9
Age (years)		
median (range)	78 (59–100)	78 (59–100)
Tumor size (cm)		
median (range)	2.0 (0.8–6.0)	2.0 (0.8–6.0)
Stage at presentation		
I	10	9
II	8	8
III	1	1
IV	2	1

For immunohistochemical analysis, 21 samples were obtained for VEGFR-2 and 19 samples for endostatin. The specimens of two patients were consumed and were not available for endostatin staining. The protocol for immunohistochemistry and statistical analysis is described in detail in articles IV and V. One section of each tumor was analyzed, and the staining pattern was recorded. The sample was considered positive irrespective of the location of the positive reaction that is whether in the tumor cells, stromal cells, or intratumoral vascular structures. The results were scored by two researches (TB and HK). The intensity of the immunoreaction was recorded as negative (-), low (+), or strong (++).

The study was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa. The Ministry of Health and Social Affairs granted permission to collect the patient data, and the National Authority for Medicolegal Affairs granted permission to collect tissue samples for study purposes.

RESULTS

EPIDEMIOLOGICAL-CLINICAL STUDY (STUDY I)

INCIDENCE

Based on the Registry data, the age-adjusted incidence of MCC (/100,000) in Finland is 0.11 for men and 0.12 for women, when adjusted to the world standard population. When the European standard population is used as the reference population, the incidence figures are 0.19 and 0.20, and for the US 2000 standard population 0.25 and 0.26, respectively. The annual number of new MCC cases has risen along with increasing awareness of this disease. After early years of recognition, the incidence estimates have become more reliable. Among men, the age-adjusted incidence rates (adjusted to the world standard population) have been fairly stable, around 0.11/ 100,000 in 1989–2008. Among women, the incidence has risen from 0.08 / 100,000 in 1989–1993 to 0.12 in 2004–2008.

TREATMENT OF DISEASE AT PRESENTATION

More than half of the patients (55%) with a known primary tumor site had a re-excision after a diagnostic excision or biopsy excision. The surgical margins varied from 0.5 to 3 cm. Histological clear margins (at least 1mm) were achieved in the majority of cases. In 12.2% of the cases a cumulative minimum margin ≥20 mm was achieved, but in 20.4% of the patients the margin status remained positive. Further operations were withheld either due to the patient's general condition, advanced age or unawareness of the malignant nature of the disease. 24 patients received post-operative radiotherapy to the primary tumor bed. The majority of the 155 patients who presented with localized Stage I-II disease were treated with marginal excision and 22 (14%) received post-operative radiotherapy to the tumor bed (Table 9).

The treatment of the draining lymph node basin was uncommon in this retrospective study. In the majority of patients with Stage I-II disease (92.3%), the draining lymph node basin was not evaluated. The first SNB to a MCC patient

in Finland was performed in 2001, and during 2001–2004, a total of 11 patients with clinically localized disease underwent SNB; one of these patients eventually had complete lymph node dissection due to positive SNB. Two other patients with Stage I-II disease at presentation underwent evacuation: one due to failed sentinel node lymphoscintigraphy, and the other due to the assumed aggressive nature of the disease.

Complete lymph node clearance was performed to four of the 18 patients (22.2%) with Stage III disease, and 8 patients received radiotherapy to the lymph node area.

RECURRENCE

The disease recurred in 59 (38%) of the patients with Stage I-II disease, after a median time of 6 months (range 1–61 mo). The relation of excision margin and radiotherapy to recurrence is presented in Table 9. The most common site (59%) for recurrence was the primary tumor site, and in 32% it developed in the regional lymph nodes (Table 10). Three of the 10 patients with negative SNB eventually hade a recurrence: 2 patients at the regional lymph nodes and one patient had in-transit metastases.

Twelve (86%) of the patients with nodal disease at presentation eventually had a recurrence at a median time of 8.5 months (range 1–97 mo). Six of these patients had distant metastases and three had a nodal recurrence.

SURVIVAL

The 5-year relative survival rate of the men was 36% (95% CI 20-54%), which was significantly lower than that of the women 69% (CI 56-82%). There was no statistical difference in survival between different tumor localizations or age groups. Although there was no significant difference in the survival rates for Stage I and Stage II disease, 68% (CI 53-82%) and 67% (CI 44-87%), respectively, the tumors of localized stage showed better prognosis compared to Stage III and Stage IV disease with 16% and 0% survival rates, respectively. There was a significant difference in survival between women and men with Stage II disease (Table 11).

Table 9. Treatment of the primary lesion and subsequent recurrences in Merkel cell carcinoma patients presenting with clinically local disease (Stage I-II) with known primary tumor (n=155)

Excision margin status	**No. of patients** (100%)	**Recurrence** No. of patients (%)			
		Only local	Nodal (+/- local)	Distant (+/- local or nodal)	Overall
Intralesional exc.	27	7 (26%)	6 (22%)	1 (4%)	14 (52%)
Intralesional exc. +RT	6	0	2 (33%)	0	2 (33%)
Marginal exc.	81	10 (12%)	10 (12%)	8 (10%)	28 (35%)
Marginal exc. +RT	13	0	2 (15%)	2 (15%)	4 (31%)
Wide exc.	18	2 (11%)	4 (22%)	1 (6%)	7 (39%)
Wide Exc +RT	2	0	0	1 (50%)	1 (50%)
Unknown	7	2 (29%)	1 (14%)	0	3 (43%)
Unknown+RT	1	0	0	0	0
Total	155	21 (14%)	25 (16%)	13 (8%)	59 (38%)

exc. = excision
RT = radiotherapy

Table 10. Site of first recurrence among the 59 patients with Stage I-II disease with a recurrence.

	Number of patients	%	Median time months (range)
Local	35	59.3	3 (1-61)
In-transit	3	5.1	7 (4-34)
Regional lymph node	19	32.2	8 (2-56)
Distant	2	3.4	9 and 27

Table 11. Five-year relative survival ratio (%) among 180* Merkel cell carcinoma patients, with 95% confidence interval, by sex and stage (closing date Dec. 31, 2007).

	Men	Women	All
Stage I	57.7 (30.5–85.6)	71.5 (55.0-–86.6)	68.7 (54.0–81.8)
Stage II	28.5 (7.0–60.4)	88.0 (56.7–114)	67.0 (44.3–88.6)
Stage III	0	28.7 (4.4–66.2)	16.5 (2.7–43.7)
Stage IV	0	0	0
All	35.7 (20.3–53.5)	69.4 (56.0–81.9)	59.2 (49.0-70.0)

* One 100-year-old patient was dropped because the population life table did not go beyond the age of 99 years.

(LYMPHO)VASCULAR INVASION IN MERKEL CELL CARCINOMA (STUDY II)

Vascular invasion was observed in 93% of the samples. LVI was the most common finding seen in 90% of the tumors. Although the tumors without vascular invasion were smaller than those with invasion, intravascular tumor embolus was found even in the smallest tumor (0.3 cm) of our study. Vascular invasion, either lymphovascular or blood vascular, was observed also in all patients with nodal or distant metastatic disease.

The vascular invasion status and development of later metastases of these Stage I and II cases is presented in Table 12. None of the patients without detected vascular invasion developed lymph node or distant metastases, nor died of MCC during the follow-up time (mean 5.2 years in this subgroup).

Table 12. Development of regional or distant metastases in cases with only local disease at the time of diagnosis (N= 111)

	LVI+, BVI+ N= 26	LVI+, BVI- N= 73	LVI-, BVI+ N= 3	LVI-, BVI- N= 9
Regional lymph node	3 (11.5%)	14 (19.2%)	0	0
Regional and distant	2 (7.7%)	2 (2.7%)	0	0
Distant only	1 (3.8%)	3 (4.1%)	1 (33.3%)	0
Total	6/ 26	19/ 73	1/ 3	0/ 9

LVI + = lymphovascular invasion detected
LVI – = lymphovascular invasion not detected
BVI + = blood vascular invasion detected
BVI – = blood vascular invasion not detected

MERKEL CELL POLYOMA VIRUS (STUDY III)

A total of 91 (79.8%) of the 114 carcinomas investigated had MCPyV DNA, as detected by quantitative PCR. Quantitative PCR analysis of all 22 control tissue samples was negative for MCPyV DNA, suggesting that the presence of viral DNA is restricted to Merkel cell carcinomas and that there was no cross-contamination between the samples.

More MCPyV-positive tumors were detected by using quantitative PCR compared to standard PCR, indicating that standard PCR is not sensitive enough to detect MCPyV DNA.

MCPyV DNA-positive cancers were located in either the upper or lower limb more often than MCPyV DNA-negative cancers. However, there was no difference between MCPyV positive and negative carcinomas in regard to sex, median age at diagnosis, histological tumor subtype, or primary tumor size. Eight patients had immunosuppression (kidney transplant, CLL, corticosteroid medication for rheumatoid arthritis) and 5 of them were MCPyV-positive.

Patients with MCPyV DNA-positive cancer had better overall survival as well as disease-specific survival than those with MCPyV DNA-negative cancer (Fig. 6A,B).

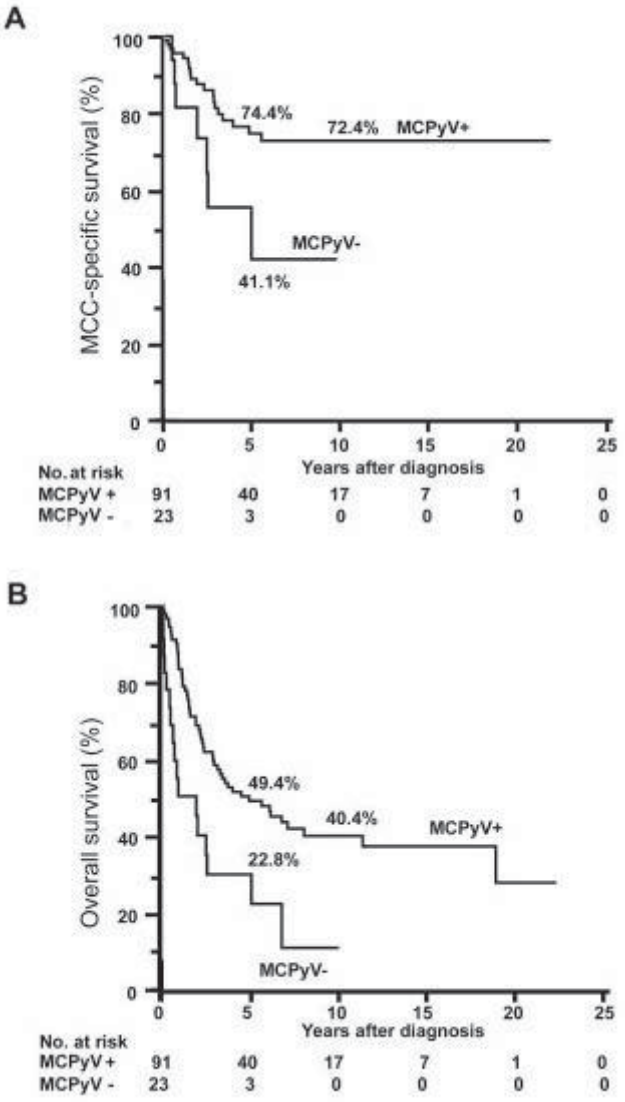


Figure 6. Kaplan-Meier analysis of the survival of patients whose tumors contained (+) Merkel cell polyoma virus DNA or lacked it (-).

A) Merkel cell carcinoma specific survival, B) overall survival

VEGFR-2 AND ENDOSTATIN (STUDIES IV,V)

Positive staining for VEGFR-2 was seen in intratumoral vessels in 80% of the samples. The tumor cells themselves showed no staining. Positive staining for endostatin was observed in tumor stromal cells and intratumoral vasculature in 42% of the samples. Two of the samples also showed dot-like perinuclear staining in the tumor cells themselves. The staining intensity of these samples is presented in Table 13.

The expression of VEGFR-2 correlated more strongly to tumor size than to development of metastases. Endostatin correlated negatively, and the correlation was again stronger with tumor size than with metastases. The simultaneous expression and non-expression of VEGFR-2 and endostatin was further analyzed. The tumors expressing only VEGFR-2 tended to be larger than those expressing also endostatin.

Table 13. Staining intensity, tumor size and possible metastasis (++ strong, + low, - negative, 0 = sample not available)

Sample no.	VEGFR-2	Endostatin	Tumor size (cm)	Metastasis
1	++	-	3.0	no
2	+	-	1.0	no
3	+	+	1.0	no
4	-	-	0.8	no
5	+	0	1.0	no
6	-	+	1.0	no
7	+	+	4.0	no
8	++	+	1.4	no
9	-	+	2.0	yes
10	+	-	6.0	yes
11	+	-	1.5	yes
12	+	-	3.0	no
13	-	-	1.2	no
14	+	0	4.0	yes
15	+	-	2.0	no
16	++	-	2.0	yes
17	++	+	1.5	yes
18	++	-	4.0	yes
19	++	+	3.0	no
20	+	+	1.5	yes
21	+	-	3.0	no

DISCUSSION

EPIDEMIOLOGICAL-CLINICAL STUDY (STUDY I)

Merkel cell carcinoma is a rare malignancy, and so far there are only a few studies on its epidemiology. It is also a relatively newly found disease; MCC was first described less than 40 years ago (Toker 1972). Some reports show an increase in MCC incidence (Hodgson 2005), but whether this is genuine increase in incidence or merely due to increased awareness of MCC and improved diagnostics is still unclear.

The Finnish Cancer Registry maintains a nationwide database of all cancers in Finland, and has a high coverage. For this study, all MCC cases recorded in the Registry were searched, and an annual incidence of 0.11 and 0.12 /100,000 was found for men and women, respectively. These figures are lower than those reported from the USA (Agelli et al. 2003). In the American cancer database, SEER, the number of HIV-positive men is overrepresented (Agelli et al. 2003, Kaae et al. 2010), and this might explain the difference in incidence compared to countries with fewer HIV-infected people. Instead, the incidence of MCC in Finland resembles more closely that of the other Nordic countries (Hussain et al. 2010, Kaae et al. 2010).

Unlike in many other studies, there are more female than male patients with MCC in Finland. The large proportion of female patients in this series may be partly due to the sharp increase in Merkel cell carcinoma incidence after the age of 70 years, and the fact that the life span of Finnish men is approximately 7 years shorter than that of Finnish women (Statistics Finland 2009). The incidence rates by age groups are nevertheless more similar in men and women.

Most of the studies on MCC are based on the diagnosis of MCC found in the database, and thus are not histopathologically confirmed. To our knowledge, this is the first nationwide study on MCC with diagnoses reconfirmed from the available original histological samples. In this series, 12 cases were identified in which the diagnosis of MCC could not be confirmed. However, it is more likely that an equal number of MCC patients are diagnosed with some other name of malignancy, for example, anaplastic lymphoma, especially during the early years when pathologists were not yet so aware of this disease. The introduction of immunohistochemical diagnostic methods in has improved the recognition of MCC.

As in other studies, the stage at presentation was the most significant prognostic factor also in this study. However, nodal staging was mostly based on clinical evaluation only in this retrospective series, and in some cases the nodal status

was not addressed. Allen et al. (2005) have shown that up to 25% of patients with clinically negative nodes were found to have a nodal disease after histopathological evaluation, and the incidence of clinically occult nodal disease was not associated with the size of the tumor. It is therefore likely that some of the patients who were evaluated as Stage I or Stage II, might have been Stage III if histopathological nodal evaluation had been performed. The survival of patients with Stage I and II disease was nevertheless the same, and it was clearly better than that of patients with clinically detected nodal or distant metastases. The most striking difference in survival was noted between men and women with Stage II disease. Women had a better prognosis than men, also in overall survival. Women tend to seek medical help earlier than men, so this might be one explanation, although there was not a statistical difference in tumor sizes at presentation between men and women. Compared to the observations of Allen at al. (2005), our survival rates were generally lower, but this may reflect the difference between a specialized cancer center and a hospital not so familiar with this uncommon disease.

To date, there is no clear consensus on the surgical margins. Most recommendations propose surgical margins of 1–2 cm, whereas some studies indicate that the width of the margins does not influence locoregional control (Gillenwater et al. 2001, Jabbour et al. 2007). Deep margins are emphasized in only a few recommendations.

In our patient series consisting of cases from a long time period, the treatment schemes were heterogeneous. 55% of the patients had undergone reoperation after diagnostic biopsy. In six cases, the margin remained positive even after the last re-excision. In this present study, extra benefit was not found from wide margins (≥2cm compared to at least 1mm), but an intralesional excision was more often followed by a local recurrence. This result supports the importance of histologically clear margins. The role of radiotherapy is also debated. In this study, none of the patients with Stage I–II disease who had received postoperative radiotherapy (n=22) had a local recurrence, so this finding favors post-operative radiotherapy to the primary tumor bed.

(LYMPHO)VASCULAR INVASION IN MERKEL CELL CARCINOMA (STUDY II)

Various rates of vascular invasion in MCC are reported, but most of the observations have been based on HE-slides only (Mott et al. 2004, Llombart et al. 2005, Feinmesser et al. 2004). The use of immunohistochemistry and endothelial cell specific markers increases the sensitivity of detecting vascular structures in MCC, as has been shown in other types of cancer as well (Kingston et al. 2007, van den Eynden et al. 2006, Lim et al. 2008).

Furthermore, in the previous studies on MCC and vascular invasion, no distinction was made between lymphovascular and blood vascular structures. When panvascular markers, such as CD31 or CD34 only are used, some of the lymphatic structures may not be visualized, as these markers are more sensitive to blood vessels than to lymphatic vessels (Podgrabinska et al. 2002).

(Lympho)vascular invasion has been proposed as a prognostic factor in MCC (Andea et al. 2008, Ng et al. 2008). Also in the present study, the patients whose tumor samples were negative for both lymphovascular and blood vascular invasion seemed to have a better prognosis. But the very high frequency of observed vascular invasion in this study, even though only one slide per patient was examined, reduces its value as a useful prognostic tool; multiple samples and sections of each tumor have to be examined to ensure that no invasion exists, thus making it unfeasible and unreliable in a clinical setting.

In malignant melanoma, lymphatic invasion is strongly associated with sentinel lymph node metastases (Niakosari et al. 2008, Doeden et al. 2009), whereas metastasis to lymph nodes is rare in thin melanomas (Lens et al. 2002). Therefore SNB is recommended only for melanomas that are more than 1 mm in depth, or when adverse features, such as ulceration or high mitotic frequency, are present. In malignant melanoma, lymphatic invasion seems to occur more frequently in the later stages of the disease (Niakosari et al. 2008). In MCC, on the other hand, lymphovascular invasion is very common and can be detected already in very small (0.3cm) MCC tumors, as seen in this study. Therefore a cutoff point for SNB, as used in the case of malignant melanoma, might not be applicable in MCC.

MERKEL CELL POLYOMA VIRUS STUDY (STUDY III)

In this study cohort, 114 Merkel cell carcinoma samples were analyzed, and MCPyV was detected in 80% of them. This is slightly higher than the proportion of MCPyV DNA-positive tumors (72.3%) in five earlier studies combined (Feng et al. 2008, Kassem et al. 2008, Foulongne et al. 2008, Becker 2009, Garneski et al. 2009). The prevalence of MCPyV in MCC varies considerably in different populations. In a study of North American patients, 69% of Merkel cell carcinoma specimens were positive for MCPyV, compared to 24% in Australian patients (Garneski et al. 2009).

Also the PCR method used for detecting MCPyV may influence the frequency of MCPyV. In this study, the LT3 primer set detected MCPyV more efficiently than the LT1 or VP1 sets, possibly because the LT3 primer set generates a shorter DNA fragment than the other two primer sets. This is consistent with two previous studies (Kassem et al. 2008, Foulongne et al. 2008). We found that quantitative PCR, which included the use of fluorescein-labeled probes and had a short amplicon length, was the most sensitive method for detecting MCPyV DNA in our study, since we used archival paraffin-embedded tissue, which very likely contained substantially fragmented DNA as the starting material. However, all strains of MCPyV may not be detectable with any one of these methods, and the prevalence of MCPyV might be even higher.

There are several observations that MCPyV infection is causally related to Merkel cell carcinogenesis. The MCPyV genome is frequently and clonally integrated in Merkel cell carcinoma, and there is evidence that MCPyV infection and integration occurs before clonal expansion of the tumor cells (Feng et al. 2008). In this study, clinical factors associated with the presence of MCPyV DNA were identified, lending support to the hypothesis that MCPyV has a role in the pathogenesis of Merkel cell carcinoma. The molecular mechanism explaining how MCPyV might contribute to the pathogenesis of Merkel cell carcinoma is still unclear.

It is not known when or how MCPyV infection occurs. The transmission route of other polyomaviruses is also unknown. The present study showed that Merkel cell carcinomas located on the limbs contained MCPyV DNA statistically significantly more often than did tumors located on the trunk. This finding suggests that MCPyV may be transmitted by physical contact.

In this study, the MCPyV DNA-positive cancers tended to have less frequent nodal metastases at the time of diagnosis, and were associated with better Merkel cell carcinoma-specific and overall survival compared with MCPyV DNA-negative cancers. This finding was emphasized by the fact that it was detected in an elderly study population that is likely to have numerous competing causes of death.

This study has a limitation. 45% of the Merkel cell carcinoma patients identified from the Finnish Cancer Registry were excluded for various reasons, mostly because

tumor tissue was not available for testing, so this may have caused possible selection bias. However, the median age at diagnosis, the sex distribution, and survival of the excluded patients did not differ statistically significantly from those of the study subjects, which suggests that exclusion of these patients did not create a major bias.

VEGFR-2 AND ENDOSTATIN (STUDIES IV,V)

In Study IV, VEGFR-2 expression was analyzed in the MCC samples, and a high frequency of expression was noted in the intratumoral vascular structures, but the MCC tumor cells did not express VEGFR-2. There is only one previous study on VEGFR-2 expression in MCC (Brenner et al.2008). In that study positive staining of the MCC tumor cells was found in 88% of the MCC samples. Immunochemistry is susceptible to many inconsistencies including antibody specificity and sensitivity, and variable staining conditions, which might explain the differences in staining patterns (Ghosh et al. 2008).

VEGFR-2 expression has been studied in other cancers with varying degrees of expression. In colorectal cancer patients, positive immunostaining for VEGFR-2 in tumor vessels was found in 92% of the samples (Okita et al. 2009). In medullary thyroid carcinoma, 91% of the samples stained positively (Capp et al. 2010). In non-small cell lung cancer (NSCLC), VEGFR-2 expression in tumor cells was observed in 93% of the samples, and most vessels also expressed VEGF-2 (Bonnessen et al. 2009). In another study on NSCLL, VEGFR-2 was detected in 54% of the tumor samples, and a relation to poorer survival was noted (Carrillo-de Santa Pau et al. 2009). An association between VEGFR-2 expression and poor outcome was shown also in breast cancer patients (Ghosh et al. 2008). The present study showed a positive correlation between expression of VEGFR-2 and tumor size. The relation to survival or to patient characteristics was not analyzed in this study.

In Study V, expression of endostatin was analyzed in 19 samples, and in 42% of them, positive staining was observed in tumor stromal cells or in the intratumoral vessels. Similar levels of expression have been demonstrated in non-small cell lung carcinoma (64%) (Iizasa et al. 2004) and in osteosarcoma (33%) (Kim et al 209). A higher expression of endostatin (93%) was shown in thyroid cancer (Hoffman et al. 2008). The typical pattern for endostatin is diffuse expression in the tumor stroma as well as in the tumor vasculature (Sund et al. 2009), as seen also in the samples of the present study.

In this study, a negative correlation was found between endostatin expression and tumor size. The simultaneous expression of VEGFR-2 and endostatin was also examined in this study. Tumors positive for VEGFR-2 and concurrently negative for endostatin seemed to be larger than the ones expressing both VEGFR-2 and

endostatin. This finding is consistent with the direct inhibitory activity of endostatin on VEGF-induced angiogenesis (Kim et al. 2002).

Contrary to the present results, a positive correlation with tumor size was found in osteosarcomas (Kim et al. 2009). A high expression of endostatin was associated with poor prognosis in hepatocellular carcinoma (Hu et al. 2005). This may seem paradoxical, since endostatin is considered to be an inhibitor of angiogenesis. In human bladder cancers expressing angioinhibitors a rapid progression was observed after removal of the primary tumor (Beecken et al. 2008). This may be explained by the sudden lack of antiangiogenic molecules produced by the primary tumor as a consequence of the surgical removal of the tumor. In the present study, both of the two tumors expressing dot-like perinuclear endostatin staining later developed metastases. The study sample was too small to allow drawing any conclusions on the prognostic significance of endostatin expression in MCC.

Anti-angiogenic therapies have been introduced for treating some cancers, (Hurwitz et al. 2004, Miller et al. 2007, Sandler et al. 2006, Escudier et al. 2007, Llovet et al. 2008). Preliminary results have been published with pazopanib, a tyrosine kinase inhibitor against several receptors, among them VEGFR-2, in MCC (Davids et al. 2009). Our findings suggest that inhibiting angiogenesis could be an option for treating MCC.

CONCLUSIONS

In Finland, MCC is a rare disease of elderly people, with incidence rates similar to those in other Nordic countries. Women have a significantly better survival than men. During recent decades, the diagnostic accuracy of MCC has improved and treatment options have developed, although some controversies still remain. Our results support the concept of treating MCC with margin-negative excision and radiotherapy to the tumor bed to reduce local recurrence.

The finding of a high frequency of lymphovascular invasion reduces its value as a prognostic factor, but emphasizes the role of SNB even in very small primary MCC tumors.

We also found that MCPyV DNA is frequently present at detectable levels in Merkel cell carcinomas and that MCPyV infection is associated with clinical outcome. Confirmation of MCPyV as a contributing factor to the pathogenesis of Merkel cell carcinoma might provide novel options for future therapeutic strategies.

The study demonstrated a correlation between VEGFR-2 and endostatin with tumor size, which suggests that antiangiogenic therapy might be one option for MCC. Based on these results, VEGFR-2 and endostatin have no prognostic relevance in the development of later metastases.

It remains to be seen in future studies whether increased awareness of this disease, its improved diagnostics, more radical surgery, possible radiotherapy and a novel therapeutic regimen will improve the survival of MCC patients in Finland.

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REFERENCES

Achen MG, Jeltsch M, Kukk E, Mäkinen T, Vitali A, Wilks AF, Alitalo K, Stacker SA. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Proc Natl Acad Sci USA 1998;95:548-53.

Achen MG, Stacker SA. Molecular control of lymphatic metastasis. Ann N Y Acad Sci 2008;1131:225-34.

Agelli M, Clegg LX. Epidemiology of primary Merkel cell carcinoma in the United States. J Am Acad Dermatol 2003;49:832-41.

AJCC 2010. Merkel cell. In: American Joint Committee on Cancer. AJCC Cancer Staging Manual. 7th ed. Springer, New York, NY; 2010:318-9

Albores-Saavedra J, Batich K, Chable-Montero F, Sagy N, Schwartz AM, Henson DE. Merkel cell carcinoma demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol 2010: 37;20-27.

Allen PJ, Bowne WB, Jaques DP, Brennan MF, Busam K, Coit DG. Merkel cell carcinoma: prognosis and treatment of patients from a single institution. J Clin Oncol 2005;23:2300-9.

Allen PJ, Zhang ZF, Coit DG. Surgical management of Merkel cell carcinoma. Ann Surg 1999;229:97-105.

Andea AA, Coit DG, Amin B, Busam KJ. Merkel cell carcinoma: histologic features and prognosis. Cancer 2008;113:2549-58.

Andres C, Belloni B, Puchta U, Sander CA, Flaig MJ. Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors. J Cutan Pathol 2010:37;28-34.

Bajetta E, Celio L, Platania M, Lo Vullo S, Patuzzo R, Maurichi A, Santinami M. Single-institution series of early-stage Merkel cell carcinoma: long-term outcomes in 95 patients managed with surgery alone. Ann Surg Oncol 2009;16:2985-93.

Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D. MC Polyomavirus

Is Frequently Present in Merkel Cell Carcinoma of European Patients. J Invest Dermatol 2009;129:248-50.

Beecken WD, Engl T, Jonas D, Blaheta RA. Expression of angiogenesis inhibitors in human bladder cancer may explain rapid metastatic progression after radical cystectomy. Int J Mol Med 2009;23:261-6.

Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401-10.

Bichakjian CK, Lowe L, Lao CD, Sandler HM, Bradford CR, Johnson TM, Wong SL. Merkel cell carcinoma: critical review with guidelines for multidisciplinary management. Cancer 2007;110:1-12.

Blezinger P, Wang J, Gondo M, Quezada A, Mehrens D, French M, Singhal A, Sullivan S, Rolland A, Ralston R, Min W. Systemic inhibition of tumor growth and tumor metastases by intramuscular administration of the endostatin gene. Nat Biotechnol 1999;17:343-8.

Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature 1997;390:404-7.

Bonnesen B, Pappot H, Holmstav J, Skov BG. Vascular endothelial growth factor A and vascular endothelial growth factor receptor 2 expression in non-small cell lung cancer patients: relation to prognosis. Lung Cancer. 2009;66:314-8.

Bordea C, Wojnarowska F, Millard PR, Doll H, Welsh K, Morris PJ. Skin cancers in renal-transplant recipients occur more frequently than previously recognized in a temperate climate. Transplantation 2004;77:574-9.

Boyer JD, Zitelli JA, Brodland DG, D'Angelo G. Local control of primary Merkel cell carcinoma: review of 45 cases treated with Mohs micrographic surgery with and without adjuvant radiation. J Am Acad Dermatol 2002;47:885-92.

Boyle F, Pendlebury S, Bell D. Further insights into the natural history and management of primary cutaneous neuroendocrine (Merkel cell) carcinoma. Int J Radiat Oncol Biol Phys 1995;31:315-23.

Brenner B, Sulkes A, Rakowsky E, Feinmesser M, Yukelson A, Bar-Haim E, Katz A, Idelevich E, Neuman A, Barhana M, Fenig E. Second neoplasms in patients with Merkel cell carcinoma. Cancer 2001;91:1358-62.

Brissett AE, Olsen KD, Kasperbauer JL, Lewis JE, Goellner JR, Spotts BE, Weaver AL, Strome SE. Merkel cell carcinoma of the head and neck: a retrospective case series. Head Neck 2002;24:982-8.

Brown HA, Sawyer DM, Woo T. Intraepidermal Merkel cell carcinoma with no dermal involvement. Am J Dermatopathol 2000;22:65-9.

Brown JA, Smoller BR. Merkel cell carcinoma: what is it, what will it do and where will it go? What role should the pathologist play in reporting this information?. J Cutan Pathol 2009;36:924-7.

Brunner M, Thurnher D, Pammer J, Geleff S, Heiduschka G, Reinisch CM, Petzelbauer P, Erovic BM. Expression of VEGF-A/C, VEGF-R2, PDGF-alpha/beta, c-kit, EGFR, Her-2/Neu, Mcl-1 and Bmi-1 in Merkel cell carcinoma. Mod Pathol 2008;21:876-84.

Buell JF, Trofe J, Hanaway MJ, Beebe TM, Gross TG, Alloway RR, First MR, Woodle ES. Immunosuppression and Merkel cell cancer. Transplant Proc 2002;34:1780-1.

Cabanas RM. An approach for the treatment of penile carcinoma. Cancer 1977;39:456-66.

Calder KB, Smoller BR. New insights into Merkel cell carcinoma. Adv Anat Pathol 2010;17:155-61.

Carrillo-de Santa Pau E, Carrillo Arias F, Caso Pelaez E, Muguruza Trueba I, Sánchez Hernández I, Muñoz Molina GM, Moreno Balsalobre R, Sacristán López S, Gómez-Pinillos A, Toledo Lobo Mdel V. Prognostic significance of the expression of vascular endothelial growth factors A, B, C, and D and their receptors R1, R2, and R3 in patients with nonsmall cell lung cancer. Cancer Invest 2010 May;28:393-8.

Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2002;2:563-72.

Compton CC, Regauer S, Seiler GR, Landry DB. Human Merkel cell regeneration in skin derived from cultured keratinocyte grafts. Lab Invest 1990;63:233-41.

Cueni LN, Detmar M. The lymphatic system in health and disease. Lymphat Res Biol. 2008;6:109-22.

Das S, Skobe M. Lymphatic vessel activation in cancer. Ann N Y Acad Sci 2008;1131:235-41.

Davids MS, Charlton A, Ng SS, Chong ML, Laubscher K, Dar M, Hodge J, Soong R, Goh BC. Response to a novel multitargeted tyrosine kinase inhibitor pazopanib in metastatic Merkel cell carcinoma. J Clin Oncol 2009;27:e97-100.

de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 1992;255:989-91.

Doeden K, Ma Z, Narasimhan B, Swetter SM, Detmar M, Dadras SS. Lymphatic invasion in cutaneous melanoma is associated with sentinel lymph node metastasis. J Cutan Pathol 2009;36:772-80.

Duncavage EJ, Zehnbauer BA, Pfeifer JD. Prevalence of Merkel cell polyomavirus in Merkel cell carcinoma. Mod Pathol 2009;22:516-21.

Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW. Merkel cell carcinoma and HIV infection. Lancet 2002;359:497-8.

Escudier B, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, Chevreau C, Filipek M, Melichar B, Bajetta E, Gorbunova V, Bay JO, Bodrogi I, Jagiello-Gruszfeld A, Moore N; AVOREN Trial investigators. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 2007;370:2103-11.

Fang LC, Lemos B, Douglas J, Iyer J, Nghiem P. Radiation monotherapy as regional treatment for lymph node-positive Merkel cell carcinoma. Cancer 2010;116:1783-90.

Feinmesser M, Halpern M, Kaganovsky E, Brenner B, Fenig E, Hodak E, Sulkes J, Okon E. C-Kit expression in primary and metastatic Merkel cell carcinoma. Am J Dermatopathol 2004;26:458-62.

Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 2008;319:1096-100.

Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669-76.

Ferringer T, Rogers HC, Metcalf JS. Merkel cell carcinoma in situ. J Cutan Pathol 2005;32:162-5.

Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol 1992;3:65-71.

Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182-6.

Folkman J. What is the evidence that tumors are angiogenesis dependent?. J Natl Cancer Inst 1990;82:4-6.

Foulongne V, Kluger N, Dereure O, Brieu N, Guillot B, Segondy M. Merkel cell polyomavirus and Merkel cell carcinoma, France. Emerg Infect Dis 2008;14:1491-3.

Frigerio B, Capella C, Eusebi V, Tenti P, Azzopardi JG. Merkel cell carcinoma of the skin: the structure and origin of normal Merkel cells. Histopathology 1983;7:229-49.

Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet 1971;1:1253-7.

Garneski KM, DeCaprio JA, Nghiem P. Does a new polyomavirus contribute to Merkel cell carcinoma?. Genome Biol 2008;9:228.

Garneski KM, Warcola AH, Feng Q, Kiviat NB, Leonard JH, Nghiem P. Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. J Invest Dermatol 2009;129:246-8.

Ghosh S, Sullivan CA, Zerkowski MP, Molinaro AM, Rimm DL, Camp RL, Chung GG. High levels of vascular endothelial growth factor and its receptors (VEGFR-1, VEGFR-2, neuropilin-1) are associated with worse outcome in breast cancer. Hum Pathol 2008;39:1835-43.

Gillenwater AM, Hessel AC, Morrison WH, Burgess M, Silva EG, Roberts D, Goepfert H. Merkel cell carcinoma of the head and neck: effect of surgical excision and radiation on recurrence and survival. Arch Otolaryngol Head Neck Surg 2001;127:149-54.

Goessling W, McKee PH, Mayer RJ. Merkel cell carcinoma. J Clin Oncol 2002;20:588-98.

Gonzales RJ, Padhya TA, Cherpelis BS, Prince MD, Aya-ay ML, Sondak VK, Cruse CW, Zager JS. The surgical management of primary and metastatic Merkel cell carcinoma. Curr Probl Cancer 2010;34:77-96.

Goudsmit J, Wertheim-van Dillen P, van Strien A, van der Noordaa J. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. J Med Virol 1982;10:91-9.

Gould VE, Lee I, Hammar SP. Neuroendocrine skin carcinoma coexpressing cytokeratin and neurofilament proteins. Ultrastruct Pathol 1985;9:83-90.

Grim M, Halata Z. Developmental origin of avian Merkel cells. Anat Embryol (Berl) 2000;202:401-10.

Guler-Nizam E, Leiter U, Metzler G, Breuninger H, Garbe C, Eigentler TK. Clinical course and prognostic factors of Merkel cell carcinoma of the skin. Br J Dermatol 2009;161:90-4.

Gupta SG, Wang LC, Penas PF, Gellenthin M, Lee SJ, Nghiem P. Sentinel lymph node biopsy for evaluation and treatment of patients with Merkel cell carcinoma: The Dana-Farber experience and meta-analysis of the literature. Arch Dermatol 2006;142:685-90.

Hanly AJ, Elgart GW, Jorda M, Smith J, Nadji M. Analysis of thyroid transcription factor-1 and cytokeratin 20 separates merkel cell carcinoma from small cell carcinoma of lung. J Cutan Pathol 2000;27:118-20.

Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang LC, Peñas PF, Nghiem P. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. J Am Acad Dermatol 2008;58:375-81.

Henness S, Vereecken P. Management of Merkel tumours: an evidence-based review. Curr Opin Oncol 2008;20:280-6.

Hill AD, Brady MS, Coit DG. Intraoperative lymphatic mapping and sentinel lymph node biopsy for Merkel cell carcinoma. Br J Surg 1999;86:518-21.

Hodgson NC. Merkel cell carcinoma: changing incidence trends. J Surg Oncol 2005;89:1-4.

Hoffmann S, Wunderlich A, Lingelbach S, Musholt PB, Musholt TJ, von Wasielewski R, Zielke A. Expression and secretion of endostatin in thyroid cancer. Ann Surg Oncol 2008;15:3601-8.

Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E, Huang T, Radziejewski C, Bailey K, Fandl JP, Daly T, Wiegand SJ, Yancopoulos GD, Rudge JS. VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci U S A 2002;99:11393-8.

Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. Cell Signal 2007;19:2003-12.

Howard RA, Dores GM, Curtis RE, Anderson WF, Travis LB. Merkel cell carcinoma and multiple primary cancers. Cancer Epidemiol Biomarkers Prev 2006;15:1545-9.

Hu TH, Huang CC, Wu CL, Lin PR, Liu SY, Lin JW, Chuang JH, Tai MH. Increased endostatin/collagen XVIII expression correlates with elevated VEGF level and poor prognosis in hepatocellular carcinoma. Mod Pathol 200;18:663-72.

Hurwitz H. Integrating the anti-VEGF-A humanized monoclonal antibody bevacizumab with chemotherapy in advanced colorectal cancer. Clin Colorectal Cancer 2004;4 Suppl 2:S62-8.

Hussain SK, Sundquist J, Hemminki K. Incidence trends of squamous cell and rare skin cancers in the Swedish national cancer registry point to calendar year and age-dependent increases. J Invest Dermatol 2010;130:1323-8.

Iizasa T, Chang H, Suzuki M, Otsuji M, Yokoi S, Chiyo M, Motohashi S, Yasufuku K, Sekine Y, Iyoda A, Shibuya K, Hiroshima K, Fujisawa T. Overexpression of collagen XVIII is associated with poor outcome and elevated levels of circulating serum endostatin in non-small cell lung cancer. Clin Cancer Res 2004;10:5361-6.

Jabbour J, Cumming R, Scolyer RA, Hruby G, Thompson JF, Lee S. Merkel cell carcinoma: assessing the effect of wide local excision, lymph node dissection, and radiotherapy on recurrence and survival in early-stage disease--results from a review of 82 consecutive cases diagnosed between 1992 and 2004. Ann Surg Oncol 2007;14:1943-52.

Jiang M, Abend JR, Johnson SF, Imperiale MJ. The role of polyomaviruses in human disease. Virology 2009;384:266-73.

Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen, I, Kukk E, Saksela O, Kalkkinen N, Alitalo K. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. EMBO 1996;15:290-98.

Junquera L, Torre A, Vicente JC, Garcia-Consuegra L, Fresno MF. Complete spontaneous regression of Merkel cell carcinoma. Ann Otol Rhinol Laryngol 2005;114:376-80.

Kaae J, Hansen AV, Biggar RJ, Boyd HA, Moore PS, Wohlfahrt J, Melbye M. Merkel cell carcinoma: Incidence, mortality, and risk of other cancers. J Natl Cancer Inst 2010;102:793-801.

Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 2003;21:60-5.

Kahn HJ, Bailey D, Marks A (a). Monoclonal antibody D2-40, a new marker of lymphatic endothelium, reacts with Kaposi's sarcoma and a subset of angiosarcomas. Mod Pathol 2002;15:434-40.

Kahn HJ, Marks A (b). A new monoclonal antibody, D2-40, for detection of lymphatic invasion in primary tumors. Lab Invest 2002;82:1255-7.

Kaipainen A, KorhonenJ, Mustonen T, van Hinsbergh VW, Fang GH, Dumont D, Breitman M, Alitalo K. Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. Proc Natl Acad Sci USA 1995;92:3566-70.

Kassem A, Schopflin A, Diaz C, Weyers W, Stickeler E, Werner M, Zur Hausen A. Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the VP1 gene. Cancer Res 2008;68:5009-13.

Katoh O, Tauchi H, Kawaishi K, Kimura A, Satow Y. Expression of the vascular endothelial growth factor (VEGF) receptor gene, KDR, in hematopoietic cells

and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. Cancer Res 1995;55:5687-92.

Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. PLoS Pathog 2009;5:e1000363.

Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 1989;246:1309-12.

Kim HS, Lim SJ, Park YK. Anti-angiogenic factor endostatin in osteosarcoma. APMIS. 2009;117:716-23.

Kim I, Ryan AM, Rohan R, Amano S, Agular S, Miller JW, Adamis AP. Constitutive expression of VEGF, VEGFR-1, and VEGFR-2 in normal eyes. Invest Ophthalmol Vis Sci 1999;40:2115-21.

Kingston EF, Goulding H, Bateman AC. Vascular invasion is underrecognized in colorectal cancer using conventional hematoxylin and eosin staining. Dis Colon Rectum 2007;50:1867-72.

Klagsbrun M, D'Amore PA. Vascular endothelial growth factor and its receptors. Cytokine Growth Factor Rev 1996;7:259-70.

Koljonen V, Bohling T, Granhroth G, Tukiainen E. Merkel cell carcinoma: a clinicopathological study of 34 patients. Eur J Surg Oncol 2003;29:607-10.

Koljonen V, Haglund C, Tukiainen E, Bohling T. Neuroendocrine differentiation in primary Merkel cell carcinoma--possible prognostic significance. Anticancer Res 2005;25:853-8.

Koljonen V, Kukko H, Pukkala E, Sankila R, Böhling T, Tukiainen E, Sihto H, Joensuu H. Chronic lymphocytic leukaemia patients have a high risk of Merkel-cell polyomavirus DNA-positive Merkel-cell carcinoma. Br J Cancer 2009;101:1444-7.

Koljonen V, Kukko H, Tukiainen E, Böhling T, Sankila R, Joensuu H, Pukkala E. Second cancers following the diagnosis of Merkel cell carcinoma: A nationwide cohort study. Cancer Epidemiol 2010;34:62-5.

Koljonen V, Kukko H, Tukiainen E, Böhling T, Sankila R, Pukkala E, Sihto H, Joensuu H, Kyllönen L, Mäkisalo H. Incidence of Merkel cell carcinoma in renal transplant recipients. Nephrol Dial Transplant 2009;24:3231-5.

Krasagakis K, Tosca AD. Overview of Merkel cell carcinoma and recent advances in research. Int J Dermatol 2003;42:669-76.

Langley RGB, Mihm MC, Sober AJ. Clinical presentation: Melanoma. In book: Sober AJ et Haluska FG (eds.) Atlas of Clinical Oncology: Skin Cancer. American Cancer Society, BC Becker Inc, Hamilton 2001; 49-59.

Lanoy E, Costagliola D, Engels EA. Skin cancers associated with HIV infection and solid-organ transplantation among elderly adults. Int J Cancer 2010;126:1724-31.

Lanoy E, Dores GM, Madeleine MM, Toro JR, Fraumeni JF,Jr, Engels EA. Epidemiology of nonkeratinocytic skin cancers among persons with AIDS in the United States. AIDS 2009;23:385-93.

LeBoit PE, Burg G, Weedon D, Sarasin A. (Eds.): World Health Organization Classification of tumours. Pathology and genetics of skin tumours. IARC press: Lyon 2006: 272-3.

Lemos BD, Storer BE, Iyer JG, Phillips JL, Bichakjian CK, Fang LC, Johnson TM, Liegeois-Kwon NJ, Otley CC, Paulson KG, Ross MI, Yu SS, Zeitouni NC, Byrd DR, Sondak VK, Gershenwald JE, Sober AJ, Nghiem P. Pathologic nodal evaluation improves prognostic accuracy in Merkel cell carcinoma: Analysis of 5823 cases as the basis of the first consensus staging system. J Am Acad Dermatol 2010;63:751-61.

Lens MB, Dawes M, Newton-Bishop JA, Goodacre T. Tumour thickness as a predictor of occult lymph node metastases in patients with stage I and II melanoma undergoing sentinel lymph node biopsy. Br J Surg 2002;89:1223-7.

Leonard JH, Ramsay JR, Kearsley JH, Birrell GW. Radiation sensitivity of Merkel cell carcinoma cell lines. Int J Radiat Oncol Biol Phys 1995;32:1401-7.

Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306-9.

Lewis KG, Weinstock MA, Weaver AL, Otley CC. Adjuvant local irradiation for Merkel cell carcinoma. Arch Dermatol 2006;142:693-700.

Lim CS, Alexander-Sefre F, Allam M, Singh N, Aleong JC, Al-Rawi H, Jacobs IJ. Clinical value of immunohistochemically detected lymphovascular space invasion in early stage cervical carcinoma. Ann Surg Oncol 2008;15:2581-8.

Ling Y, Yang Y, Lu N, You QD, Wang S, Gao Y, Chen Y, Guo QL. Endostar, a novel recombinant human endostatin, exerts antiangiogenic effect via blocking VEGF-induced tyrosine phosphorylation of KDR/Flk-1 of endothelial cells. Biochem Biophys Res Commun 2007;361:79-84.

Llombart B, Monteagudo C, Lopez-Guerrero JA, Carda C, Jorda E, Sanmartín O, Almenar S, Molina I, Martín JM, Llombart-Bosch A. Clinicopathological and immunohistochemical analysis of 20 cases of Merkel cell carcinoma in search of prognostic markers. Histopathology 2005;46:622-34.

Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators study group. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.

Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol 2009;21:154-65.

Lunder EJ, Stern RS. Merkel-cell carcinomas in patients treated with methoxsalen and ultraviolet A radiation. N Engl J Med 1998;339:1247-8.

MacDonald IC, Groom AC, Chambers AF. Cancer spread and micrometastasis development: quantitative approaches for in vivo models. Bioessays 2002;24:885-93.

Major EO, Mourrain P, Cummins C. JC virus-induced owl monkey glioblastoma cells in culture: biological properties associated with the viral early gene product. Virology 1984;136:359-67.

Maricich SM, Wellnitz SA, Nelson AM, Lesniak DR, Gerling GJ, Lumpkin EA, Zoghbi HY. Merkel cells are essential for light-touch responses. Science 2009;324:1580-2.

McKee P, Calonje E, Granter S (a). Pathology of the skin with clinical correlations. 3rd Edition. Elsevier Mosby 2005; 1-36.

McKee P, Calonje E, Granter S (b). Pathology of the skin with clinical correlations. 3rd Edition. Elsevier Mosby 2005; 1230-7.

Medina-Franco H, Urist MM, Fiveash J, Heslin MJ, Bland KI, Beenken SW. Multimodality treatment of Merkel cell carcinoma: case series and literature review of 1024 cases. Ann Surg Oncol 2001;8:204-8.

Meeuwissen JA, Bourne RG, Kearsley JH. The importance of postoperative radiation therapy in the treatment of Merkel cell carcinoma. Int J Radiat Oncol Biol Phys 1995;31:325-31.

Mehrany K, Otley CC, Weenig RH, Phillips PK, Roenigk RK, Nguyen TH. A meta-analysis of the prognostic significance of sentinel lymph node status in Merkel cell carcinoma. Dermatol Surg 2002;28:113-7.

Menaker GM, Chiu DS. Basal cell carcinoma. In book: Sober AJ, Haluska FG (edit.) Atlas of Clinical Oncology: Skin Cancer. American Cancer Society, BC Becker Inc, Hamilton 2001; 60-71.

Mertz KD, Junt T, Schmid M, Pfaltz M, Kempf W. Inflammatory monocytes are a reservoir for Merkel cell polyomavirus. J Invest Dermatol 2010;130:1146-51.

Messina JL, Reintgen DS, Cruse CW, Rappaport DP, Berman C, Fenske NA, Glass LF. Selective lymphadenectomy in patients with Merkel cell (cutaneous neuroendocrine) carcinoma. Ann Surg Oncol 1997;4:389-95.

Mikkilineni R, Weinstock MA. Epidemiology. In book: Sober AJ et Haluska FG (edit.) Atlas of Clinical Oncology: Skin Cancer. American Cancer Society, BC Becker Inc, Hamilton 2001; 1-15.

Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, Davidson NE. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357:2666-76.

Mills LA, Durrani AJ, Watson JD. Merkel cell carcinoma in South East Scotland, 1993-2003. Surgeon 2006;4:133-8.

Minton TJ. Contemporary Mohs surgery applications. Curr Opin Otolaryngol Head Neck Surg 2008;16:376-80.

Miosge N, Sasaki T, Timpl R. Angiogenesis inhibitor endostatin is a distinct component of elastic fibers in vessel walls. FASEB J 1999;13:1743-50.

Mohs FE, Guyer MF. Pre-excisional fixation of tissues in the treatment of cancer in rats. Cancer Res. 1941;1:49-51.

Moll I, Zieger W, Schmelz M. Proliferative Merkel cells were not detected in human skin. Arch Dermatol Res 1996;288:184-7.

Monaco MC, Jensen PN, Hou J, Durham LC, Major EO. Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection. J Virol 1998;72:9918-23.

Morrison KM, Miesegaes GR, Lumpkin EA, Maricich SM. Mammalian Merkel cells are descended from the epidermal lineage. Dev Biol 2009;336:76-83.

Morrison WH, Peters LJ, Silva EG, Wendt CD, Ang KK, Goepfert H. The essential role of radiation therapy in securing locoregional control of Merkel cell carcinoma. Int J Radiat Oncol Biol Phys 1990;19:583-91.

Mortier L, Mirabel X, Fournier C, Piette F, Lartigau E. Radiotherapy alone for primary Merkel cell carcinoma. Arch Dermatol 2003;139:1587-90.

Mott RT, Smoller BR, Morgan MB. Merkel cell carcinoma: a clinicopathologic study with prognostic implications. J Cutan Pathol 2004;31:217-23.

NCCN National Comprehensive Cancer Network. Clinical Practical Guidelines in Oncology: Merkel Cell Carcinoma. 2010:1-19.

Nghiem P, McKee PH, Haynes HA. Merkel Cell (Cutaneous Neuroendocrine) Carcinoma. In book: Sober AJ et Haluska FG (edit.) Atlas of Clinical Oncology: Skin Cancer. American Cancer Society, BC Becker Inc, Hamilton 2001; 127-41.

Ng L, Beer TW, Murray K. Vascular density has prognostic value in Merkel cell carcinoma. Am J Dermatopathol 2008;30:442-5.

Niakosari F, Kahn HJ, McCready D, Ghazarian D, Rotstein LE, Marks A, Kiss A, From L. Lymphatic invasion identified by monoclonal antibody D2-40, younger age, and ulceration: predictors of sentinel lymph node involvement in primary cutaneous melanoma. Arch Dermatol 2008;144:462-7.

Nyberg P, Xie L, Kalluri R. Endogenous inhibitors of angiogenesis. Cancer Res 2005;65:3967-79.

O'Connor WJ, Brodland DG. Merkel cell carcinoma. Dermatol Surg 1996;22:262-7.

Okita NT, Yamada Y, Takahari D, Hirashima Y, Matsubara J, Kato K, Hamaguchi T, Shirao K, Shimada Y, Taniguchi H, Shimoda T. Vascular endothelial growth factor receptor expression as a prognostic marker for survival in colorectal cancer. Jpn J Clin Oncol. 2009;39:595-600.

O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 1994;79:315-28.

O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997;88:277-85.

Ott MJ, Tanabe KK, Gadd MA, Stark P, Smith BL, Finkelstein DM, Souba WW. Multimodality management of Merkel cell carcinoma. Arch Surg 1999;134:388,92; discussion 392-3.

Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet 1971;1:1257-60.

Pectasides D, Pectasides M, Economopoulos T. Merkel cell cancer of the skin. Ann Oncol 2006;17:1489-95.

Penn I, First MR. Merkel's cell carcinoma in organ recipients: report of 41 cases. Transplantation 1999;68:1717-21.

Pepper MS. Lymphangiogenesis and tumor metastasis: myth or reality?. Clin Cancer Res 2001;7:462-8.

Pfeifer T, Weinberg H, Brady MS. Lymphatic mapping for Merkel cell carcinoma. J Am Acad Dermatol 1997;37:650-1.

Podgrabinska S, Braun P, Velasco P, Kloos B, Pepper MS, Skobe M. Molecular characterization of lymphatic endothelial cells. Proc Natl Acad Sci U S A 2002;99:16069-74.

Popp S, Waltering S, Herbst C, Moll I, Boukamp P. UV-B-type mutations and chromosomal imbalances indicate common pathways for the development of Merkel and skin squamous cell carcinomas. Int J Cancer 2002;99:352-60.

Poulsen M. Merkel-cell carcinoma of the skin. Lancet Oncol 2004;5:593-9.

Poulsen M, Rischin D. Merkel cell carcinoma--current therapeutic options. Expert Opin Pharmacother 2003;4:2187-92.

Poulsen M, Round C, Keller J, Tripcony L, Veness M. Factors influencing relapse-free survival in Merkel cell carcinoma of the lower limb - a review of 60 cases. Int J Radiat Oncol Biol Phys 2010;76:393-7.

Presta LG, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, Winkler M, Ferrara N. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 1997;57:4593-9.

Prewett M, Huber J, Li Y, Santiago A, O'Connor W, King K, Overholser J, Hooper A, Pytowski B, Witte L, Bohlen P, Hicklin DJ. Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. Cancer Res 1999;59:5209-18.

Pulitzer MP, Amin BD, Busam KJ. Merkel cell carcinoma: review. Adv Anat Pathol 2009;16:135-44.

Raica M, Ribatti D. Targeting tumor lymphangiogenesis: an update. Curr Med Chem. 2010;17:698-708.

Ramchandran R, Karumanchi SA, Hanai J, Alper SL, Sukhatme VP. Cellular actions and signaling by endostatin. Crit Rev Eukaryot Gene Expr 2002;12:175-91.

Rao NG. Review of the role of radiation therapy in the management of Merkel cell carcinoma. Curr Probl Cancer 2010;34:108-17.

Rashid K, Ng R, Mastan A, Sager D, Hirschman R. Accelerated growth of skin carcinoma following fludarabine therapy for chronic lymphocytic leukemia. Leuk Lymphoma 2005;46:1051-5.

Ribatti D. Endogenous inhibitors of angiogenesis: a historical review. Leuk Res 2009;33:638-44.

Riou-Gotta MO, Fournier E, Danzon A, Pelletier F, Levang J, Mermet I, Blanc D, Humbert P, Aubin F. Rare skin cancer: a population-based cancer registry descriptive study of 151 consecutive cases diagnosed between 1980 and 2004. Acta Oncol 2009;48:605-9.

Robinson JK. Squamous cell carcinoma. In book: Sober AJ et Haluska FG (edit.) Atlas of Clinical Oncology: Skin Cancer. American Cancer Society, BC Becker Inc, Hamilton 2001; 72-84.

Runger TM. How different wavelengths of the ultraviolet spectrum contribute to skin carcinogenesis: the role of cellular damage responses. J Invest Dermatol 2007;127:2103-5.

Sais G, Admella C, Soler T. Spontaneous regression in primary cutaneous neuroendocrine (Merkel cell) carcinoma: a rare immune phenomenon?. J Eur Acad Dermatol Venereol 2002;16:82-3.

Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355:2542-50.

Sarnaik AA, Zager JS, Cox LE, Ochoa TM, Messina JL, Sondak VK. Routine omission of sentinel lymph node biopsy for merkel cell carcinoma <= 1 cm is not justified. J Clin Oncol 2010;28:e7.

Schmid C, Beham A, Feichtinger J, Aubock L, Dietze O. Recurrent and subsequently metastasizing Merkel cell carcinoma in a 7-year-old girl. Histopathology 1992;20:437-9.

Schwartz JL, Griffith KA, Lowe L, Wong SL, McLean SA, Fullen DR, Lao CD, Hayman JA, Bradford CR, Rees RS, Johnson TM, Bichakjian CK. Features predicting sentinel lymph node positivity in Merkel cell carcinoma. J Clin Oncol 2011 (epub ahead of print)

Seiffert K, Granstein RD. Neuropeptides and neuroendocrine hormones in ultraviolet radiation-induced immunosuppression. Methods 2002;28:97-103.

Senchenkov A, Barnes SA, Moran SL. Predictors of survival and recurrence in the surgical treatment of merkel cell carcinoma of the extremities. J Surg Oncol 2007;95:229-34.

Shields JD, Borsetti M, Rigby H, Harper SJ, Mortimer PS, Levick JR, Orlando A, Bates DO. Lymphatic density and metastatic spread in human malignant melanoma. Br J Cancer 2004;90:693-700.

Shields JD, Fleury ME, Yong C, Tomei AA, Randolph GJ, Swartz MA. Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. Cancer Cell. 2007;11:526-38.

Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, Chang Y. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci U S A 2008;105:16272-7.

Sidhu GS, Chandra P, Cassai ND. Merkel cells, normal and neoplastic: an update. Ultrastruct Pathol 2005;29:287-94.

Skelton HG, Smith KJ, Hitchcock CL, McCarthy WF, Lupton GP, Graham JH. Merkel cell carcinoma: analysis of clinical, histologic, and immunohistologic features of 132 cases with relation to survival. J Am Acad Dermatol 1997;37:734-9.

Sleeman JP, Krishnan J, Kirkin V, Baumann P. Markers for the lymphatic endothelium: in search of the holy grail? Microsc Res Tech. 2001 Oct 15;55:61-9.

Sleeman JP, Thiele W. Tumor metastasis and the lymphatic vasculature. Int J Cancer 2009;125:2747-56.

Smith KJ, Skelton HG,3rd, Holland TT, Morgan AM, Lupton GP. Neuroendocrine (Merkel cell) carcinoma with an intraepidermal component. Am J Dermatopathol 1993;15:528-33.

Stokes JB, Graw KS, Dengel LT, Swenson BR, Bauer TW, Slingluff CL Jr, Ledesma EJ. Patients with Merkel cell carcinoma tumors < or = 1.0 cm in diameter are unlikely to harbor regional lymph node metastasis. J Clin Oncol 2009;27:3772-7.

Sund M, Hamano Y, Sugimoto H, Sudhakar A, Soubasakos M, Yerramalla U, Benjamin LE, Lawler J, Kieran M, Shah A, Kalluri R. Function of endogenous inhibitors of angiogenesis as endothelium-specific tumor suppressors. Proc Natl Acad Sci U S A. 2005;102:2934-9.

Sund M, Kalluri R. Tumor stroma derived biomarkers in cancer. Cancer Metastasis Rev. 2009;28:177-83.

Szeder V, Grim M, Halata Z, Sieber-Blum M. Neural crest origin of mammalian Merkel cells. Dev Biol 2003;253:258-63.

Tai PT, Yu E, Winquist E, Hammond A, Stitt L, Tonita J, Gilchrist J. Chemotherapy in neuroendocrine/ Merkel cell carcinoma of the skin: case series and review of 204 cases. J Clin Oncol 2000;18:2493-9.

Tang CK, Toker C. Trabecular carcinoma of the skin: an ultrastructural study. Cancer 1978;42:2311-21.

Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. Experience in Finland. Acta Oncol 1994;33:365-9.

Terman BI, Carrion ME, Kovacs E, Rasmussen BA, Eddy RL, Shows TB. Identification of a new endothelial cell growth factor receptor tyrosine kinase. Oncogene 1991;6:1677-83.

Toker C. Trabecular carcinoma of the skin. Arch Dermatol 1972;105:107-10.

Tolstov YL, Pastrana DV, Feng H, Becker JC, Jenkins FJ, Moschos S, Chang Y, Buck CB, Moore PS. Human Merkel cell polyomavirus infection II. MCV is a common human infection that can be detected by conformational capsid epitope immunoassays. Int J Cancer 2009;125:1250-6.

Van den Eynden GG, Van der Auwera I, Van Laere SJ, Colpaert CG, van Dam P, Dirix LY, Vermeulen PB, Van Marck EA. Distinguishing blood and lymph vessel invasion in breast cancer: a prospective immunohistochemical study. Br J Cancer 2006;94:1643-9.

Van Keymeulen A, Mascre G, Youseff KK, Harel I, Michaux C, De Geest N, Szpalski C, Achouri Y, Bloch W, Hassan BA, Blanpain C. Epidermal progenitors give rise to Merkel cells during embryonic development and adult homeostasis. J Cell Biol 2009;187:91-100.

Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis. Semin Cancer Biol 1999;9:211-20.

Veness MJ. Merkel cell carcinoma: improved outcome with the addition of adjuvant therapy. J Clin Oncol 2005;23:7235-6.

Warner RE, Quinn MJ, Hruby G, Scolyer RA, Uren RF, Thompson JF. Management of merkel cell carcinoma: the roles of lymphoscintigraphy, sentinel lymph node biopsy and adjuvant radiotherapy. Ann Surg Oncol 2008;15:2509-18.

Wasserberg N, Schachter J, Fenig E, Feinmesser M, Gutman H. Applicability of the sentinel node technique to Merkel cell carcinoma. Dermatol Surg 2000;26:138-41.

Wong SY, Haack H, Crowley D, Barry M, Bronson RT, Hynes RO. Tumor-secreted vascular endothelial growth factor-C is necessary for prostate cancer lymphangiogenesis, but lymphangiogenesis is unnecessary for lymph node metastasis. Cancer Res 2005;65:9789-98.

Wood JM, Bold G, Buchdunger E, Cozens R, Ferrari S, Frei J, Hofmann F, Mestan J, Mett H, O'Reilly T, Persohn E, Rösel J, Schnell C, Stover D, Theuer A, Towbin H, Wenger F, Woods-Cook K, Menrad A, Siemeister G, Schirner M, Thierauch KH, Schneider MR, Drevs J, Martiny-Baron G, Totzke F. PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. Cancer Res 2000;60:2178-89.

Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, Steinberg SM, Chen HX, Rosenberg SA. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003;349:427-34.

Yiengpruksawan A, Coit DG, Thaler HT, Urmacher C, Knapper WK. Merkel cell carcinoma. Prognosis and management. Arch Surg 1991;126:1514-9.

Youker SR. Merkel cell carcinoma. Adv Dermatol 2003;19:185-205.

Zhan FQ, Packianathan VS, Zeitouni NC. Merkel cell carcinoma: a review of current advances. J Natl Compr Canc Netw 2009;7:333-9.

zur Hausen H. Novel human polyomaviruses--re-emergence of a well known virus family as possible human carcinogens. Int J Cancer 2008;123:247-50.